NTP
National Toxicology Program
U.S. Department of Health and Human Services

NTP Technical Report on the Toxicology and Carcinogenesis Studies of *N,N*-Dimethyl-*p*-Toluidine (CAS No. 99-97-8) in F344/N Rats and B6C3F1/N Mice (Gavage Studies)

NTP TR 579

SEPTEMBER 2012

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF *N*,*N*-DIMETHYL-*p*-TOLUIDINE
(CAS NO. 99-97-8)

IN F344/N RATS AND B6C3F1/N MICE
(GAVAGE STUDIES)

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September 2012

NTP TR 579

NIH Publication No. 12-5921

National Institutes of Health
Public Health Service
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOREWORD

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Background

N,*N*-Dimethyl-*p*-toluidine is used in hardening dental materials and bone cements. We studied the effects of *N*,*N*-dimethyl-*p*-toluidine on male and female rats and mice to identify potential toxic or cancer-related hazards.

Methods

We deposited solutions containing *N*,*N*-dimethyl-*p*-toluidine in corn oil through a tube directly into the stomach to groups of 50 male and female rats and mice five days per week for two years. Animals received 6, 20, or 60 milligrams of *N*,*N*-dimethyl-*p*-toluidine per kilogram body weight. Control animals received corn oil with no chemical added by the same method. At the end of the study, tissues from more than 40 sites were examined for every animal.

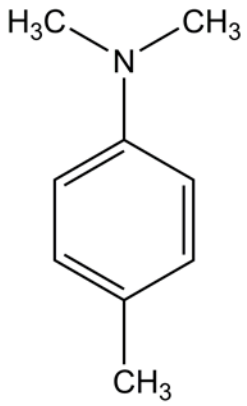
Results

In all four studies, the incidences of liver tumors were greater in animals receiving *N*,*N*-dimethyl-*p*-toluidine than in the control groups. These tumors were hepatocellular carcinomas and adenomas in both rats and mice, plus hepatoblastomas in male and female mice. Male and female rats administered *N*,*N*-dimethyl-*p*-toluidine had cancers of the nose (transitional epithelial adenomas). Male rats also had slightly increased rates of thyroid gland tumors. Female mice exposed to *N*,*N*-dimethyl-*p*-toluidine also had increased incidences of lung tumors (alvelolar/bronchiolar adenomas and carcinomas) and of forestomach tumors.

Conclusions

We conclude that *N*,*N*-dimethyl-*p*-toluidine caused cancers of the liver and nose in male and female rats, cancer of the liver in male and female mice, and cancers of the lung and forestomach in female mice. Thyroid gland tumors in male rats may also have been related to exposure to *N*,*N*-dimethyl-*p*-toluidine.

ABSTRACT



_N,N_-DIMETHYL-_p_-TOLUIDINE

CAS No. 99-97-8

Chemical Formula: $C_9H_{13}N$ Molecular Weight: 135.21

Synonyms: *N*,*N*-dimethyl-4-methylaniline; dimethyl-4-toluidine; dimethyl-*p*-toluidine; *N*,*N*-dimethyl-*p*-tolylamine; *p*-(dimethylamino)toluene; *p*-methyl-*N*,*N*-dimethylaniline; *N*,*N*,4-trimethylaniline; *p*,*N*,*N*-trimethylaniline; *N*,*N*,4-trimethylbenzenamine

N,*N*-Dimethyl-*p*-toluidine was nominated for toxicology and carcinogenesis studies by the National Cancer Institute based on the potential for human exposure through its use in dental materials and bone cements and the lack of toxicity and carcinogenicity data. Male and female F344/N rats and B6C3F1/N mice were administered *N*,*N*-dimethyl-*p*-toluidine (greater than 99% pure) in corn oil by gavage for 3 months or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and *Escherichia coli*, mouse peripheral blood, and mouse and rat liver.

3-MONTH STUDY IN RATS

Groups of 10 male and 10 female rats were administered 0, 62.5, 125, 250, 500, or 1,000 mg *N*,*N*-dimethyl-*p*-toluidine/kg body weight in corn oil by gavage, 5 days per week for 14 weeks. Additional groups of 10 male and 10 female rats (clinical pathology study) were administered the same doses, 5 days per week for 25 days. On day 88, blood was collected from core study rats for hemoglobin and methemoglobin analyses only. All 1,000 mg/kg male and female rats and one 500 mg/kg male rat died by study day 3. Mean body weights of all surviving dosed groups of males and females were significantly less than those of the vehicle controls. Clinical findings associated with exposure to *N*,*N*-dimethyl-*p*-toluidine included cyanosis, abnormal breathing, and lethargy in groups administered 250 mg/kg or greater.

Methemoglobinemia appeared to be the primary hematologic toxic response, and many other lesions could be explained as secondary to methemoglobin formation including Heinz body formation; a macrocytic, hypochromic, responsive anemia; and increased hematopoietic cell proliferation in the spleen and bone marrow. In general, hematologic changes were dose-related and occurred at both evaluated timepoints in all dosed groups. Anemia was evidenced by decreases in hematocrit values, hemoglobin concentrations, and erythrocyte counts; erythrocyte macrocytosis was characterized by increases in mean cell volume and mean cell hemoglobin values; erythrocyte hypochromia was evidenced by decreases in mean cell hemoglobin concentration values; and an erythropoietic response to the anemia

was characterized by substantially increased reticulocyte and nucleated erythrocyte counts. Liver weights of all surviving dosed groups of males and females were significantly greater than those of the vehicle controls. Kidney weights of all surviving dosed groups of females were significantly greater than those of the vehicle controls. There were significant decreases in left cauda epididymis and left epididymis weights in 250 mg/kg males. There was a dose-related decrease in the number of cycling females, with only four females in the 250 mg/kg group having regular cycles and females in the 125 and 250 mg/kg groups spending a significantly higher proportion of time in extended diestrus compared to the vehicle control group.

In the surviving groups of rats, there were significantly increased incidences of pigmentation in the liver of all dosed groups, hepatocyte hypertrophy in groups administered 125 mg/kg or greater, and hepatocyte necrosis in 62.5, 250, and 500 mg/kg females. In the olfactory epithelium of the nose, there were dose-related increases in the incidences and severities of degeneration in all dosed groups and significantly increased incidences of metaplasia in the 250 and 500 mg/kg groups. In the respiratory epithelium of the nose, there were significantly increased incidences of hyperplasia and squamous metaplasia in all of the groups administered 125 mg/kg or greater. The incidences of glandular hyperplasia of the nose were significantly increased in males and females administered 125, 250, or 500 mg/kg. In the spleen, there were significantly increased incidences of capsule fibrosis, congestion, mesothelial hypertrophy, and lymphoid follicle atrophy primarily in groups administered 125 mg/kg or greater. Hematopoietic cell proliferation and pigmentation were increased in severity in treated groups. In the kidney, there were significantly increased incidences of nephropathy (females), pigmentation (males and females), papillary necrosis (males and females), and mineralization (males). Other treatment-related lesions included inflammation of the forestomach in males, mesenteric lymph node atrophy in females, and bone marrow hyperplasia in males and females.

3-Month Study in Mice

Groups of 10 male and 10 female mice were administered 0, 15, 30, 60, 125, or 250 mg *N,N*-dimethyl-*p*-toluidine/kg body weight in corn oil by gavage, 5 days per week for 14 weeks. All 250 mg/kg male and female mice (except for one male mouse) died before day 10, and three males and two females administered 125 mg/kg died before the end of the study. The final mean body weight of 125 mg/kg males and the mean body weight gains of 125 mg/kg males and females were significantly less than those of the vehicle controls. Clinical findings associated with administration of *N,N*-dimethyl-*p*-toluidine included abnormal breathing, thinness, lethargy, cyanosis, and ruffled fur in 125 and 250 mg/kg males and females.

Methemoglobinemia appeared to be the primary hematologic toxic response; however there were less severe erythron changes compared to the 3-month study in rats. In females, no erythron changes were detected up to 125 mg/kg. In males, inconsistent and minor decreases in hematocrit values, hemoglobin concentrations, and erythrocyte counts, and increased reticulocyte counts occurred in groups administered 60 mg/kg or greater. Methemoglobin values were minimally increased in males and females administered 30 mg/kg or greater. Heinz bodies were slightly increased in 60 mg/kg females, 125 mg/kg males and females, and the one surviving 250 mg/kg male; Heinz body formation was considered secondary to methemoglobin formation. Liver weights of all dosed groups of mice were significantly greater than those of the vehicle controls.

In the surviving groups of mice, there were significantly increased incidences of bronchiolar epithelium degeneration, bronchiolar epithelium regeneration, and peribronchiolar chronic active inflammation in the lung of 125 mg/kg groups, and histiocytic infiltrates of the alveoli in 125 mg/kg females. In the nose, there were significantly increased incidences of glandular hyperplasia and olfactory epithelium metaplasia in the 125 mg/kg groups and olfactory epithelium degeneration in 60 mg/kg females and 125 mg/kg males and females. In the thymus, the incidences of thymocyte necrosis in the 125 mg/kg groups were significantly increased. In the liver, the severities of cytoplasmic vacuolization of the hepatocytes were increased in dosed groups of males and females.

2-Year Study in Rats

Groups of 50 male and 50 female rats were administered 0, 6, 20, or 60 mg *N,N*-dimethyl-*p*-toluidine/kg body weight in corn oil by gavage, 5 days per week for 104 or 105 weeks. Additional groups of 10 male and 10 female rats (clinical pathology study) were administered the same doses for 86 days. Survival of 60 mg/kg males was significantly less than that of the vehicle controls. Mean body weights of 60 mg/kg males and females were more than 10% less than those of the

vehicle controls after week 61 and week 33, respectively. Clinical findings included signs of pallor in 60 mg/kg females and hyperactivity and boxing behavior in 20 mg/kg females and 60 mg/kg males and females.

The hematology findings at the 3-month timepoint were consistent with those in the 3-month study in rats which indicated that methemoglobinemia was the primary hematologic toxic response. In the 20 and 60 mg/kg groups, there were dose-related decreases in hematocrit values, hemoglobin concentrations, and erythrocyte counts. There were similar trends toward erythrocyte macrocytosis and hypochromia and increased erythropoiesis as seen in the 3-month study. While the magnitudes of the erythron decreases were not sufficient to classify the responses as anemias, the patterns of the erythron changes were identical to those in the 3-month study.

In the liver of 60 mg/kg males and females, there were significantly increased incidences of hepatocellular carcinoma and hepatocellular adenoma or hepatocellular carcinoma (combined). Numerous nonneoplastic liver lesions occurred in dosed males and females primarily in the 20 and 60 mg/kg groups.

In the nose, there were significantly increased incidences of transitional epithelium adenoma and transitional epithelium adenoma or carcinoma (combined) in 60 mg/kg males; transitional epithelium adenoma also occurred in female rats administered 6 or 60 mg/kg. In the nose, there were significantly increased incidences of nonneoplastic lesions in the olfactory, respiratory, and transitional epithelia of dosed rats. These lesions occurred with the greatest incidence and severity in the 60 mg/kg groups. The incidences of inflammation and nerve atrophy were significantly increased in males and females administered 60 mg/kg.

There were increased incidences of follicular cell adenoma or carcinoma (combined) of the thyroid gland in all dosed groups of males, and an increased incidence of follicular cell adenoma in 20 mg/kg females.

In the spleen, there were significantly increased incidences of hematopoietic cell proliferation in all dosed groups of males and females. The incidences of congestion and mesothelial hypertrophy of the capsule were significantly increased in 60 mg/kg males and all dosed groups of females. There were also significantly increased incidences of capsular fibrosis and atrophy of the lymphoid follicle in the 60 mg/kg groups. The incidences of pigmentation were significantly increased in all dosed groups of males and in 60 mg/kg females.

In all dosed groups of female rats, there were significantly increased incidences of nephropathy. Although the incidences of this lesion were not significantly increased in dosed males, the severities increased with increasing dose in both males and females. The incidences of pigmentation of the kidney were significantly increased in all dosed groups of males and in 60 mg/kg females.

In the forestomach of males, there were significantly increased incidences of hyperplasia and ulcer in the 20 and 60 mg/kg groups and inflammation in the 60 mg/kg group. In the bone marrow of 20 and 60 mg/kg males and 60 mg/kg females, there were significantly increased incidences of hyperplasia. In the mesenteric lymph node of 20 and 60 mg/kg males, there were significantly increased incidences of histiocytic cellular infiltrates.

2-YEAR STUDY IN MICE

Groups of 50 male and 50 female mice were administered 0, 6, 20, or 60 mg *N,N*-dimethyl-*p*-toluidine/kg body weight in corn oil by gavage, 5 days per week for 105 weeks. Survival of 60 mg/kg females was significantly less than that of the vehicle control group. Mean body weights of 60 mg/kg males and females were more than 10% less than those of the vehicle controls after week 89 and week 65, respectively.

In the liver, there were significantly increased incidences of hepatocellular adenoma in 20 and 60 mg/kg females and hepatocellular carcinoma in 60 mg/kg males and all dosed female groups. The increased incidences of hepatocellular adenoma and carcinoma in the dosed groups were primarily due to increased incidences of animals with multiple hepatocellular neoplasms. The incidences of hepatoblastoma were significantly increased in 20 mg/kg males and 60 mg/kg males and females. In all dosed groups of males and females, there were significantly increased incidences of hepatocyte hypertrophy, and the incidences of eosinophilic focus were significantly increased in the 20 and 60 mg/kg males and females. There were significantly increased incidences of fatty change and necrosis in 60 mg/kg females.

In the lung of 20 and 60 mg/kg female mice, there were significantly increased incidences of alveolar/ bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined). There were also significantly increased incidences of alveolar epithelium hyperplasia in 20 mg/kg females; bronchiolar epithelium regeneration, bronchus epithelium regeneration, and bronchus

necrosis in 60 mg/kg females; and alveolar infiltrates of histiocytes in 60 mg/kg males and females.

In the forestomach of 20 and 60 mg/kg female mice, there were significantly increased incidences of squamous cell papilloma and squamous cell papilloma or carcinoma (combined). There were significantly increased incidences of epithelium hyperplasia in 20 and 60 mg/kg females, and inflammation and ulcer in 60 mg/kg females.

In the nose, there were significantly increased incidences of nonneoplastic lesions of the olfactory and respiratory epithelia in 60 mg/kg males and 20 and 60 mg/kg females. There were significantly increased incidences of nerve atrophy in 60 mg/kg males and females and of inflammation in 60 mg/kg females. The incidences of atrophy of the olfactory lobe were significantly increased in 60 mg/kg males and females. In 60 mg/kg females, the incidences of regenerative hyperplasia of the nasolacrimal duct and necrosis of the vomeronasal organ were significantly increased.

In the spleen, there was a significantly increased incidence of atrophy in 60 mg/kg females. There were also significantly increased incidences of bone marrow hyperplasia in all dosed groups of females and mesenteric lymph node atrophy in 60 mg/kg females.

GENETIC TOXICOLOGY

N,N-Dimethyl-*p*-toluidine was tested in two independent bacterial gene mutation studies; both studies gave negative results in *S. typhimurium* or *E. coli* tester strains, with and without exogenous metabolic activation. *In vivo*, no significant increases in the frequencies of micronucleated erythrocytes were observed in peripheral blood of male or female B6C3F1/N mice treated with *N,N*-dimethyl-*p*-toluidine by gavage for 3 months. Furthermore, no increases in micronucleated reticulocytes were observed in male B6C3F1/N mice treated with *N,N*-dimethyl-*p*-toluidine for 4 days. Results of DNA damage (comet) studies yielded mixed results. No increases in DNA damage (measured as percent tail DNA) were seen in liver cells or blood leukocytes of male B6C3F1/N mice administered *N,N*-dimethyl-*p*-toluidine by gavage once daily for 4 days. However, a small but significant increase in DNA damage was seen in liver cells of male Sprague-Dawley rats administered 60 mg/kg *N,N*-dimethyl-*p*-toluidine once daily for 4 days.

CONCLUSIONS

Under the conditions of these 2-year oral gavage studies, there was *clear evidence of carcinogenic activity** of *N,N*-dimethyl-*p*-toluidine in male F344/N rats based on increased incidences of hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined), and increased incidences of nasal cavity neoplasms (primarily nasal cavity transitional epithelium adenoma). The increased incidences of thyroid gland follicular cell neoplasms may have been related to treatment. There was *clear evidence of carcinogenic activity* of *N,N*-dimethyl-*p*-toluidine in female F344/N rats based on increased incidences of hepatocellular carcinoma and hepatocellular adenoma or carcinoma (combined). The occurrence of nasal cavity transitional epithelium adenoma was considered to be related to treatment. There was *clear evidence of carcinogenic activity* of *N,N*-dimethyl-*p*-toluidine in male B6C3F1/N mice based on increased incidences of hepatocellular adenoma (multiple), hepatocellular carcinoma, and hepatoblastoma. There was *clear evidence of carcinogenic activity* of *N,N*-dimethyl-*p*-toluidine in female B6C3F1/N mice based on increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma and increased incidences of alveolar/bronchiolar neoplasms (primarily adenoma). The increased incidences of forestomach squamous cell papilloma in female mice were considered to be related to treatment.

Administration of *N,N*-dimethyl-*p*-toluidine resulted in increased incidences of nonneoplastic lesions of the liver and nasal cavity in male and female rats and mice; the kidney in male and female rats; the spleen and bone marrow in male and female rats and female mice; the lung in male and female mice; the forestomach in male rats and female mice; the mesenteric lymph node in male rats and female mice; and the olfactory lobe in male and female mice.

N,N-Dimethyl-*p*-toluidine also caused hematologic toxicity and increases in methemoglobin levels in male and female rats and mice (as measured at 3 months).

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 14. A summary of the Peer Review Panel comments and the public discussion on this Technical Report appears on page 16.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of *N,N*-Dimethyl-*p*-toluidine

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Doses in corn oil by gavage	0, 6, 20, or 60 mg/kg	0, 6, 20, or 60 mg/kg	0, 6, 20, or 60 mg/kg	0, 6, 20, or 60 mg/kg
Body weights	60 mg/kg group 10% less than the vehicle control group after week 61	60 mg/kg group 10% less than the vehicle control group after week 33	60 mg/kg group 10% less than the vehicle control group after week 89	60 mg/kg group 10% less than the vehicle control group after week 65
Survival rates	37/50, 37/50, 31/50, 21/50	33/50, 42/50, 33/50, 23/50	34/50, 36/50, 31/50, 36/50	43/50, 40/50, 39/50, 32/50
Nonneoplastic effects	Liver: eosinophilic focus (11/50, 21/50, 21/50, 29/50); mixed cell focus (18/50, 17/50, 17/50, 35/50); bile duct, fibrosis (21/50, 27/50, 41/50, 42/50); bile duct, hyperplasia (40/50, 42/50, 44/50, 44/50); degeneration, cystic (4/50, 10/50, 9/50, 17/50); hepatocyte, hypertrophy (0/50, 0/50, 6/50, 31/50) Nose: glands, olfactory epithelium, dilatation (0/50, 0/49, 3/50, 49/49); glands, olfactory epithelium, hyperplasia (0/50, 2/49, 0/50, 48/49); glands, olfactory epithelium, metaplasia (0/50, 0/49, 0/50, 38/49); glands, olfactory epithelium, necrosis (0/50, 0/49, 0/50, 22/49); glands, respiratory epithelium, dilatation (13/50, 15/49, 19/50, 48/49); glands, respiratory epithelium, hyperplasia (0/50, 8/49, 8/50, 41/49); glands, respiratory epithelium, metaplasia, respiratory (29/50, 39/49, 39/50, 47/49); glands, transitional epithelium, dilatation (0/50, 0/49, 5/50, 3/49); glands, transitional epithelium, hyperplasia (0/50, 1/49, 24/50, 40/49); inflammation (35/50, 40/49, 38/50, 48/49); nerve, atrophy (0/50, 0/49, 0/50, 15/49); olfactory epithelium, degeneration (0/50, 0/49, 1/50, 47/49); olfactory epithelium, hyperplasia, basal cell (0/50, 1/49, 2/50, 38/49);	Liver: clear cell focus (7/50, 17/50, 24/50, 29/49); eosinophilic focus (18/50, 24/50, 29/50, 32/49); mixed cell focus (14/50, 20/50, 17/50, 26/49); bile duct, fibrosis (6/50, 11/50, 23/50, 27/49); bile duct, hyperplasia (10/50, 21/50, 27/50, 43/49); degeneration, cystic (0/50, 0/50, 2/50, 10/49); hepatocyte, hypertrophy (0/50, 0/50, 6/50, 22/49); hepatocyte, necrosis (0/50, 0/50, 1/50, 5/49) Nose: glands, olfactory epithelium, dilatation (0/50, 0/49, 0/50, 48/49); glands, olfactory epithelium, hyperplasia (0/50, 0/49, 4/50, 47/49); glands, olfactory epithelium, metaplasia (0/50, 0/49, 0/50, 42/49); glands, olfactory epithelium, necrosis (0/50, 0/49, 0/50, 18/49); glands, respiratory epithelium, dilatation (5/50, 12/49, 27/50, 47/49); glands, respiratory epithelium, hyperplasia (6/50, 9/49, 22/50, 45/49); glands, respiratory epithelium, metaplasia, respiratory (17/50, 33/49, 44/50, 47/49); glands, transitional epithelium, dilatation (0/50, 0/49, 0/50, 9/49); glands, transitional epithelium, hyperplasia (0/50, 4/49, 12/50, 24/49); inflammation (23/50, 24/49, 22/50, 45/49); nerve, atrophy (0/50, 0/49, 0/50, 4/49);	Liver: eosinophilic focus (25/50, 30/50, 39/50, 43/50); hepatocyte, hypertrophy (1/50, 9/50, 11/50, 16/50) Nose: glands, olfactory epithelium, dilatation (4/49, 11/50, 7/50, 48/50); glands, olfactory epithelium, hyperplasia (4/49, 9/50, 7/50, 49/50); glands, olfactory epithelium, metaplasia, respiratory (5/49, 5/50, 6/50, 48/50); glands, respiratory epithelium, dilatation (17/49, 19/50, 13/50, 41/50); glands, respiratory epithelium, metaplasia, respiratory (2/49, 2/50, 2/50, 10/50); nerve, atrophy (2/49, 7/50, 4/50, 42/50); olfactory epithelium, metaplasia, respiratory (10/49, 10/50, 5/50, 49/50); olfactory epithelium, necrosis (1/49, 3/50, 3/50, 8/50) Lung: alveolus, infiltration cellular, histiocyte (1/50, 2/50, 2/50, 10/50) Olfactory lobe: atrophy (0/38, 1/43, 0/39, 5/34)	Liver: eosinophilic focus (20/50, 18/50, 45/50, 38/50); fatty change (1/50, 0/50, 0/50, 8/50); hepatocyte, hypertrophy (0/50, 11/50, 10/50, 17/50); necrosis (1/50, 8/50, 4/50, 10/50) Lung: alveolar epithelium, hyperplasia (2/50, 3/50, 8/50, 2/50); alveolus, infiltration cellular, histiocyte (1/50, 0/50, 0/50, 7/50); bronchiole, epithelium, regeneration (0/50, 0/50, 0/50, 5/50); bronchus, epithelium, regeneration (0/50, 0/50, 0/50, 5/50); bronchus, necrosis (0/50, 0/50, 0/50, 5/50) Forestomach: epithelium, hyperplasia (3/50, 5/50, 12/50, 17/50); inflammation (3/50, 4/50, 7/50, 16/50); ulcer (2/50, 2/50, 4/50, 7/50) Nose: glands, olfactory epithelium, dilatation (13/50, 14/49, 20/50, 46/50); glands, olfactory epithelium, hyperplasia (2/50, 14/49, 14/50, 50/50); glands, olfactory epithelium, metaplasia, respiratory (2/50, 5/49, 7/50, 44/50); glands, respiratory epithelium, dilatation (10/50, 17/49, 15/50, 33/50); glands, respiratory epithelium, hyperplasia (0/50, 2/49, 12/50, 13/50); glands, respiratory epithelium, metaplasia, respiratory (0/50, 0/49, 10/50, 10/50); inflammation (3/50, 7/49, 3/50, 32/50);

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of *N,N*-Dimethyl-*p*-toluidine

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Nonneoplastic effects (continued)	Nose (continued): olfactory epithelium, metaplasia, respiratory (4/50, 9/49, 9/50, 40/49); respiratory epithelium, hyperplasia (15/50, 29/49, 32/50, 49/49); transitional epithelium, hyperplasia (1/50, 1/49, 11/50, 46/49) Spleen: capsule, fibrosis (1/50, 0/50, 2/50, 46/50); capsule, hypertrophy, mesothelium (0/50, 1/50, 3/50, 39/50); congestion (1/50, 0/50, 0/50, 39/50); hematopoietic cell proliferation (34/50, 44/50, 42/50, 44/50); lymphoid follicle, atrophy (0/50, 5/50, 2/50, 19/50); pigmentation (36/50, 48/50, 47/50, 48/50) Kidney: severity of nephropathy (1.4, 2.0, 2.5, 2.7); pigmentation (24/50, 46/50, 37/50, 44/50) Forestomach: hyperplasia (0/50, 3/50, 5/50, 11/50); inflammation (1/50, 5/50, 5/50, 7/50); ulcer (0/50, 2/50, 5/50, 6/50) Bone marrow: hyperplasia (17/50, 13/50, 28/50, 50/50) Mesenteric lymph node: infiltration cellular, histiocyte (21/50, 23/50, 30/50, 34/50)	Nose (continued): olfactory epithelium, degeneration (0/50, 0/49, 1/50, 46/49); olfactory epithelium, hyperplasia, basal cell (0/50, 0/49, 0/50, 25/49); olfactory epithelium, metaplasia, respiratory (4/50, 6/49, 1/50, 21/49); respiratory epithelium, hyperplasia (10/50, 13/49, 11/50, 41/49); transitional epithelium, hyperplasia (0/50, 1/49, 6/50, 33/49) Spleen: capsule, fibrosis (8/50, 0/50, 8/50, 41/50); capsule, hypertrophy, mesothelium (1/50, 14/50, 10/50, 16/50); congestion (0/50, 9/50, 26/50, 28/50); hematopoietic cell proliferation (32/50, 45/50, 47/50, 42/50); lymphoid follicle, atrophy (1/50, 2/50, 0/50, 28/50); pigmentation (44/50, 47/50, 47/50, 49/50) Kidney: nephropathy (28/50, 38/50, 38/50, 41/50): severity of nephropathy (1.1, 1.2, 1.2, 1.8); pigmentation (41/50, 45/50, 43/50, 49/50) Bone marrow: hyperplasia (18/50, 13/50, 18/50, 49/50)		Nose (continued): nasolacrimal duct, hyperplasia, regenerative (0/50, 0/49, 0/50, 4/50); nerve, atrophy (0/50, 0/49, 0/50, 41/50); olfactory epithelium, accumulation, hyaline droplet (2/50, 5/49, 8/50, 15/50); olfactory epithelium, metaplasia, respiratory (1/50, 6/49, 14/50, 46/50); olfactory epithelium, necrosis (0/50, 0/49, 3/50, 6/50); respiratory epithelium, hyperplasia (11/50, 15/49, 11/50, 30/50); respiratory epithelium, necrosis (0/50, 0/49, 0/50, 5/50); vomeronasal organ, necrosis (0/50, 0/49, 0/50, 4/50) Olfactory lobe: atrophy (0/27, 0/34, 0/24, 8/29) Bone marrow: hyperplasia (5/50, 14/50, 15/50, 14/49) Mesenteric lymph node: atrophy (1/49, 5/49, 5/49, 12/50) Spleen: red pulp atrophy (0/49, 0/49, 0/49, 5/50)
Neoplastic effects	Liver: hepatocellular carcinoma (0/50, 0/50, 1/50, 6/50); hepatocellular adenoma or carcinoma (0/50, 0/50, 2/50, 6/50) Nose: transitional epithelium, adenoma (0/50, 3/49, 2/50, 11/49); transitional epithelium, adenoma or carcinoma (0/50, 3/49, 2/50, 13/49)	Liver: hepatocellular carcinoma (0/50, 0/50, 0/50, 4/49); hepatocellular adenoma or carcinoma (0/50, 1/50, 1/50, 7/49) Nose: transitional epithelium, adenoma (0/50, 1/49, 0/50, 2/49)	Liver: hepatocellular adenoma, multiple (17/50, 19/50, 27/50, 26/50); hepatocellular carcinoma (22/50, 25/50, 30/50, 36/50); hepatoblastoma (1/50, 5/50, 10/50, 8/50)	Liver: hepatocellular adenoma (17/50, 19/50, 37/50, 44/50); hepatocellular carcinoma (6/50, 13/50, 18/50, 31/50); hepatoblastoma (0/50, 1/50, 0/50, 4/50) Lung: alveolar/bronchiolar adenoma (2/50, 4/50, 8/50, 12/50); alveolar/bronchiolar adenoma or carcinoma (2/50, 5/50, 9/50, 13/50)

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of *N,N*-Dimethyl-*p*-toluidine

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Neoplastic effects (continued)				Forestomach: squamous cell papilloma (1/50, 5/50, 6/50, 7/50); squamous cell papilloma or carcinoma (1/50, 6/50, 6/50, 7/50)
Equivocal effects	Thyroid gland: follicular cell adenoma or carcinoma (1/50, 2/49, 2/50, 4/49)	None	None	None
Level of evidence of carcinogenic activity	Clear evidence	Clear evidence	Clear evidence	Clear evidence
Genetic toxicology				
Bacterial gene mutations:			Negative in *S. typhimurium* strains TA97, TA98, TA100, and TA1535 with and without S9; negative in *E. coli* WP2 *uvrA*/pKM101 with and without S9	
Micronucleated erythrocytes				
Mouse peripheral blood *in vivo*:			Negative in males and females (3-month study) and males (4-day study)	
DNA damage				
Male mouse blood and liver *in vivo*:			Negative	
Male rat liver *in vivo*:			Positive	

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of evidence observed in each experiment: two categories for positive results **(clear evidence and some evidence)**; one category for uncertain findings **(equivocal evidence)**; one category for no observable effects **(no evidence)**; and one category for experiments that cannot be evaluated because of major flaws **(inadequate study)**. These categories of interpretative conclusions were first adopted in June 1983 and then revised on March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

For studies showing multiple chemical-related neoplastic effects that if considered individually would be assigned to different levels of evidence categories, the following convention has been adopted to convey completely the study results. In a study with clear evidence of carcinogenic activity at some tissue sites, other responses that alone might be deemed some evidence are indicated as “were also related” to chemical exposure. In studies with clear or some evidence of carcinogenic activity, other responses that alone might be termed equivocal evidence are indicated as “may have been” related to chemical exposure.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS
PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft NTP Technical Report on *N,N*-dimethyl-*p*-toluidine on February 8, 2012, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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Gainesville, FL

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Saskatchewan, Canada

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Michael V. Pino, D.V.M., Ph.D., Primary Reviewer
Sanofi
Bridgewater, NJ

Keith A. Soper, Ph.D.
Merck Research Laboratories
West Point, PA

SUMMARY OF PEER REVIEW PANEL COMMENTS

On February 8, 2011, the draft Technical Report on the toxicology and carcinogenesis studies of *N,N*-dimethyl-*p*-toluidine received public review by the National Toxicology Program's Technical Reports Peer Review Panel. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.K. Dunnick, NIEHS, introduced the draft Technical Report on *N,N*-dimethyl-*p*-toluidine by describing the chemical's use in dental materials and bone cements, the negative findings in genetic toxicity tests, the occurrence of hematologic toxicity and non-neoplastic lesions in the liver, nasal cavity, and the hematopoietic system in short-term studies, and neoplastic and nonneoplastic lesions in the 2-year studies. The proposed conclusions were *clear evidence* of carcinogenic activity of *N,N*-dimethyl-*p*-toluidine in male and female F344/N rats, and *clear evidence* of carcinogenic activity of *N,N*-dimethyl-*p*-toluidine in male and female B6C3F1/N mice.

Dr. Pino, the first primary reviewer, said that the studies were adequately conducted and that the dose selections for the 2-year studies were appropriate. He said that for the liver neoplasms in male rats, *clear evidence* of carcinogenicity should be based on carcinomas only, not combined with adenomas. He noted that the incidence of thyroid gland follicular adenoma in female rats was only slightly above the concurrent and historical ranges and asked whether these neoplasms were considered related to *N,N*-dimethyl-*p*-toluidine treatment or not. He noted that while the rat uterine stromal polyps and granulosa cell neoplasms and the tongue neoplasms were mentioned in the text, it was unclear if they were considered chemical-related effects. He suggested that the extended diestrus noted in female rats may be a secondary effect. Overall, he agreed with the conclusions, except for suggesting that the *clear evidence* in male rats was due to hepatocellular carcinomas, and should not be combined with adenomas.

Dr. Carpenter, the second primary reviewer, said he concurred with the calls that had been made by the staff, and that it was a very strong study. He noted that there is ample evidence for exposure to the general public, as well as occupational exposure. He felt that the presence of rare neoplasms was quite important and made the call much stronger.

Dr. Peterson, the third primary reviewer, concurred with the proposed conclusions.

Dr. Dunnick replied that the call on hepatocellular neoplasms was mainly related to the hepatocellular carcinomas, and the hepatocellular adenomas had been included because they are part of the same carcinogenic response. Regarding the thyroid gland neoplasms in the female rats, she said it was not a significant effect and was not considered to be related to the chemical. The few tongue and uterine neoplasms were noted in the results text for completeness but were not included in the overall conclusion. Dr. Dunnick explained that after consulting with experts, the staff felt that the extended diestrus indicated a potential for reproductive toxicity.

Dr. Alcorn asked when NTP considers total neoplasm incidence in making a call, when there are sometimes decreases. Dr. Dunnick said the decrease in mononuclear cell leukemia was a phenomenon seen with other nitro-aromatic compounds, and that it was discussed as a finding typical with this class of chemical.

Dr. Cattley agreed that hepatocellular carcinomas were the primary liver neoplasms in male rats, and suggested that the conclusion should reflect that. Dr. Elwell asked about the standard protocol for examining the tongue. Study pathologist, Dr. A.E. Brix, NIEHS, said that occasionally wet tissue was examined when warranted by gross examination.

Dr. Alcorn suggested corn oil was a potential confounder in any study of a chemical's carcinogenic potential and asked if NTP was planning to move away from corn oil as a delivery vehicle for lipophilic compounds. Dr. J.R. Bucher, NIEHS, said no such plan was presently in place. Dr. Anderson noted that questions have arisen about the nutritional role of the corn oil compared to the animals' diets. Dr. A.P. King-Herbert, NIEHS, explained that the NTP-2000 diet does include corn oil, and that there is a nutritional analysis of how much fat is in the diet.

Dr. Pino moved to modify the conclusion for the male rat study by striking the reference to "and hepatocellular adenoma or carcinoma (combined)." Dr. Mirsalis seconded the motion. Dr. D.E. Malarkey, NIEHS, mentioned that hepatocellular adenomas were less common in the rat compared to the mouse and they are known to

progress to carcinomas, which was the rationale for combining the neoplasms types. Dr. Carpenter added that it was his impression that this was a fairly standard way of referring to those neoplasms. Dr. Malarkey said that occasionally the reference is stated as "predominantly carcinomas." Dr. Carpenter said he would be more comfortable with that terminology.

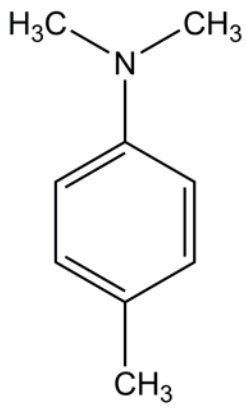
Dr. Roberts called for a vote on Dr. Pino's motion to strike the line "and hepatocellular adenoma or carcinoma (combined)." The motion failed with three yes votes and seven no votes.

Dr. Carpenter suggested retaining the original language while adding "primarily carcinomas." Dr. Malarkey suggested "(primarily carcinoma)." Drs. L.S. Birnbaum and R.C. Sills, NIEHS, noted that the original language was standard NTP language, and suggested it be retained as well.

Dr. Soper moved to accept the original language in the conclusion's first sentence and Drs. Elwell and Peterson seconded. The motion carried with eight yes votes and two no votes. Drs. Pino and Olivero voted no. Dr. Pino cited the reasons he had already stated, that the hepatocellular carcinomas were primarily responsible for the liver neoplasms in male rats and the conclusion should be reworded to state that. Dr. Olivero felt that the paragraph was not clear enough as it stood.

Dr. Carpenter moved to accept the full study conclusions as written. Dr. Peterson seconded. The panel voted in favor of the motion with eight yes votes and two no votes. Drs. Pino and Olivero voted no, for the same reasons they had stated for the prior motion.

INTRODUCTION



***N*,*N*-DIMETHYL-*p*-TOLUIDINE**

CAS No. 99-97-8

Chemical Formula: $C_9H_{13}N$ Molecular Weight: 135.21

Synonyms: *N*,*N*-dimethyl-4-methylaniline; dimethyl-4-toluidine; dimethyl-*p*-toluidine; *N*,*N*-dimethyl-*p*-tolylamine; *p*-(dimethylamino)toluene; *p*-methyl-*N*,*N*-dimethylaniline; *N*,*N*,4-trimethylaniline; *p*,*N*,*N*-trimethylaniline; *N*,*N*,4-trimethylbenzenamine

CHEMICAL AND PHYSICAL PROPERTIES

N,*N*-Dimethyl-*p*-toluidine is a colorless to brown oil with a sweet odor and a boiling point of 211° C (Mallinckrodt Baker, 1996; Verschueren, 1996). It has a log P of 2.99 (Verschueren, 1996) and is insoluble in water, miscible in ether and ethanol, and soluble in carbon tetrachloride (Lide, 1997). *N*,*N*-Dimethyl-*p*-toluidine is stable under normal use and storage conditions (Mallinckrodt Baker, 1996).

PRODUCTION, USE, AND HUMAN EXPOSURE

N,*N*-Dimethyl-*p*-toluidine is a high-production volume chemical with potential for widespread human exposure through its use in dental materials and bone cements (Hirabayashi and Imai, 2002; Hirabayashi, 2003; Timmer *et al*., 2003; Lewis *et al*., 2007a; Lewis, 2008). An estimated 1 to 10 million pounds of *N*,*N*-dimethyl-*p*-toluidine are produced per year in the United States (USEPA, 2011a). *N*,*N*-Dimethyl-*p*-toluidine is an accelerator in the redox initiator-accelerator system used commercially to cure methyl methacrylate monomers. Polymerization is rarely complete (Shintani *et al*., 1993; Stea *et al*., 1997).

Acryl resins used in dental practice are blends of poly(methyl methacrylated) particles and methyl methacrylate monomer, or copolymers of methyl methacrylate with styrene or other acrylic monomers. This blend is a slurry of high viscosity that is hardened by the free radical polymerization of the monomeric components. The hardening process is initiated by the decomposition of a small quantity of organic peroxides (1% to 3%; usually benzoyl peroxide) activated by the redox reaction with the tertiary amine (Vazquez *et al*., 1998). The tertiary amine, most often *N*,*N*-dimethyl-*p*-toluidine, is the ingredient that induces the reaction giving rise to free radicals capable of initiating polymerization of the acrylic monomers (Vazquez *et al*., 1998). Polymerization is rarely complete (Tosti *et al*., 1990).

N,N-Dimethyl-*p*-toluidine has been used in the preparation of acrylic denture materials for the past 50 years (Vazquez *et al.*, 1998). It is used as the accelerator for the cement in most of the hip and bone replacements to activate the polymerization reaction (Lewis *et al*., 2007b) at concentrations ranging from 0.7% to 2.6% (Linder, 1976; Haddad *et al*., 1996; Stea *et al*., 1997). *N,N*-Dimethyl-*p*-toluidine is found in industrial glues and artificial fingernail preparations and is used as an intermediate in dye and pesticide synthesis (Potter *et al*., 1988; Taningher *et al*., 1993; Haddad *et al*., 1996). It has a shorter setting time (11.5 minutes) than some alternative accelerators (Liso *et al*., 1997). The residual amounts of *N,N*-dimethyl-*p*-toluidine in acrylic resins have been reported to be 0.6% after storage in water for up to 15 months (Brauer *et al*., 1977). Others have detected up to 0.2% of *N,N*-dimethyl-*p*-toluidine in methyl methacrylates after long term implantation (Bösch *et al*., 1982).

The National Occupational Exposure Survey, which was conducted by the National Institute for Occupational Safety and Health (NIOSH) between 1981 and 1983, estimated that 62,720 workers were potentially exposed to *N,N*-dimethyl-*p*-toluidine in the workplace (NIOSH, 1990). There is potential for widespread human exposure to *N,N*-dimethyl-*p*-toluidine in occupational settings where bone cements, dental prostheses, industrial glues, and artificial fingernails are manufactured or used. Exposure to *N,N*-dimethyl-*p*-toluidine may be a concern because of the possible release of unreacted chemicals from polymeric composites (Kronoveter, 1977; Taningher *et al*., 1993; Haddad *et al*., 1996).

"Sniffing" glue is one possible means of exposure to *N,N*-dimethyl-*p*-toluidine (Neumark *et al*., 1998; Wu *et al*., 2008; Marsolek *et al*., 2010). *N,N*-Dimethyl-*p*-toluidine may be present in various glues at concentrations of 1% to 7% (Misiak and Scheffler, 2003; 3M, 2004).

REGULATORY STATUS

No standards or guidelines have been set by NIOSH or the Occupational Safety and Health Administration (OSHA) for occupational exposure to *N,N*-dimethyl-*p*-toluidine (NIOSH, 2007). Under the Food and Drug Administration guidelines, bone cements are classified as drugs (Brauer *et al*., 1986). *N,N*-Dimethyl-*p*-toluidine is on the United States Environmental Protection Agency High Production Challenge Program (USEPA, 2011b).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Experimental Animals

In a study conducted for the NTP, ^{14}C uniformly ring-labeled *N,N*-dimethyl-*p*-toluidine was rapidly absorbed from the gastrointestinal tract of F344 rats and B6C3F1 mice and excreted primarily in the urine as metabolites (Lovelace, 2006; Dix *et al*., 2007). Groups of three or four male rats and mice received single doses of 2.5, 25, or 250 mg/kg by gavage (in 10% aqueous Alkamuls®) or 2.5 mg/kg by intravenous (IV) injection. Female rats and mice received an oral dose of 25 mg/kg. The cumulative disposition data were similar between males receiving either 2.5 or 25 mg/kg and for female rats. Rats excreted approximately 90% of the total dose in urine and 4% in feces within 24 hours of dosing. Approximately 4% of the total dose remained in tissues and the gastrointestinal tract at the 24-hour terminal timepoint. Absorption of these doses was estimated to be at or near 100% based on comparison of oral and IV data. The cumulative disposition data for the 2.5 and 25 mg/kg doses in male mice were similar to those observed for rats. However, female mice excreted less ^{14}C (approximately 77% of the total dose) in cumulative (0- to 24-hour) urine than did male mice. The amounts excreted in feces and remaining in tissues and the gastrointestinal tract were similar between male and female mice and the lower amount of ^{14}C detected in female urine corresponded to a lower dose recovery. In male rats, excretion of ^{14}C in the urine over time was affected by dose. A lower rate of excretion of the 25 mg/kg dose was observed over the first 6 hours; however, cumulative excretion was similar to that of the 2.5 mg/kg dose by the 12-hour timepoint. Urinary excretion of the high dose (250 mg/kg) amounted to only 70% of the total dose at 24 hours after dosing. A small amount (approximately 2%) of the high dose was excreted in feces and an average of 18% remained in tissues and the gastrointestinal tract at this timepoint. Approximately 8% of this total was detected in the stomach. At 72 hours after dosing, only residual amounts (approximately 2%) of ^{14}C remained in tissues and the gastrointestinal tract in male rats treated with 250 mg/kg. This result indicated near complete absorption and excretion of the high dose over the extended holding period. *N,N*-Dimethyl-*p*-toluidine-derived radioactivity was excreted at similar rates over time in the 2.5 and 25 mg/kg male mouse dosing groups. Disposition data are not reported here for mice receiving 250 mg/kg by gavage due to acute toxicity, including mortality in the group. Less than 1% of the total

administered ^{14}C was excreted as volatiles by gavaged rats and mice. At the lower doses in rats, kidney and liver contained the highest amounts of residual ^{14}C, and with the urinary bladder, were the only tissues with a tissue:blood ratio greater than one. The amounts in blood, kidney, and liver were generally proportional to dose. In contrast to results observed at the lower doses, adipose tissue of 250 mg/kg rats contained amounts of ^{14}C similar to those observed in liver and kidney. Liver and lung contained the highest amounts of residual ^{14}C in mice. Toxicity may have contributed to the delayed gastric emptying, absorption, and excretion observed in 250 mg/kg male rats. Clinical signs of toxicity (decreased activity, piloerection, excessive blinking, and hunched posture) were observed in the rats; however, the effects were transitory. No significant vehicle effects were observed in a group of male rats receiving 250 mg/kg in corn oil, indicating that the disposition data presented here using the aqueous-based vehicle would be applicable to oral toxicity studies of *N,N*-dimethyl-*p*-toluidine using a corn oil vehicle.

Samples from these studies were analyzed by high performance liquid chromatography (HPLC) for the presence of *N,N*-dimethyl-*p*-toluidine and metabolites (Lovelace, 2006; Kim *et al*., 2007). In male rats, the major metabolite in urine was identified as *p*-(*N*-acetylhydroxyamino)hippuric acid by mass spectrometry and nuclear magnetic resonance analysis (Figure 1). Two lesser metabolites were identified as *N,N*-dimethyl-*p*-toluidine *N*-oxide and *N*-methyl-*p*-toluidine. A small amount (not quantitated) of unmetabolized *N,N*-dimethyl-*p*-toluidine was also detected in the urine. Approximately 8% of the radiolabel administered to most of the treatment groups consisted of ^{14}C-*N*-methyl-*p*-toluidine and may have contributed to the amount of the metabolite observed in rat urine. *N*-Methyl-*p*-toluidine was confirmed as a urinary metabolite of *N,N*-dimethyl-*p*-toluidine following intravenous treatment of a group of male rats with purified radiolabel. Furthermore, *N*-demethylation of *N,N*-dimethylaniline has been shown to occur in rat hepatocyte and guinea pig and rabbit tissue incubations (Gorrod and Gooderham, 1981; Sherratt and Damani, 1989) and is evident for *N,N*-dimethyl-*p*-toluidine from the large amount of *p*-(*N*-acetylhydroxyamino)hippuric acid present in the urine of rats in the current study. Exposure to *N,N*-dimethyl-*p*-toluidine is known to cause methemoglobinemia in humans, putatively via formation of *p*-methylphenylhydroxylamine (Potter *et al*., 1988). A similar metabolite (phenylhydroxylamine) of aniline is a potent inducer of methemoglobinemia in rats (Harrison and Jollow, 1987). *p*-Methylphenylhydroxylamine was not detected or identified in the current studies; however, it can be postulated to be an intermediate in the formation of *p*-(*N*-acetylhydroxyamino)-hippuric acid through *N*-methyl-*p*-toluidine. Further, the formation of *p*-methylphenylhydroxylamine may lead to covalent binding with DNA (Marques *et al*., 1997) or perhaps give rise to an imine methide or quinone imine similar to that for the hepatotoxicant, 4-hydroxyacetanilide (acetaminophen) (Peter, 1989). Metabolism data for mice were not reported by Kim *et al*. (2007) and metabolites in mouse urine were not specifically identified in the report submitted to the NTP (Lovelace, 2006). However, the information indicated that *N,N*-dimethyl-*p*-toluidine-derived metabolites were qualitatively, but not quantitatively similar between rats and mice. For instance, the major peak in mouse urine did not appear to be *p*-(*N*-acetylhydroxyamino)hippuric acid.

Humans

No data describing the *in vivo* fate of *N,N*-dimethyl-*p*-toluidine in humans were identified in the literature.

TOXICITY

Experimental Animals

LD_{50} values of *N,N*-dimethyl-*p*-toluidine in the rat are 1,650 mg/kg (oral; methemoglobinemia noted) and 1.4 mg/L (4-hour inhalation LC_{50}) (RTECS, 2011). In the New Zealand White rabbit, the dermal LD_{50} is greater than 2,000 mg/kg (RTECS, 2011). The intraperitoneal LD_{50} in the mouse is 212 mg/kg and both the 24- and 96-hour LC_{50} values in fish are 52 mg/L (Geiger *et al*., 1986; Verschueren, 1996; RTECS, 2011). The intravenous LD_{50} of *N,N*-dimethyl-*p*-toluidine in mice was 75 mg/kg (Liso *et al*., 1997).

The acute 4-hour inhalation toxicity of *N,N*-dimethyl-*p*-toluidine was assessed in male and female Sprague-Dawley rats following doses of 0.30, 0.99, 1.73, or 5.27 mg/L (ChemFirst, 1997). Clinical signs in rats exposed to 1.73 mg/L included hypoactivity, a comatose/prostrate condition, dyspnea or rapid respiration, and salivation. Nasal discharge and red material around the nose were observed in the 0.30 and 0.99 mg/L groups. Mottled lungs, red ovaries, and gas-filled gastrointestinal organs were observed in the rats exposed to 1.73 or 5.27 mg/L.

No 2-week or 3-month studies of *N,N*-dimethyl-*p*-toluidine were reported in the literature.

Humans

A 16-month-old girl (Potter *et al*., 1988) and a 5-month-old boy (Kao *et al*., 1997) developed methemoglobinemia following ingestion of 15 and 30 mL, respectively, of artificial fingernail solutions containing

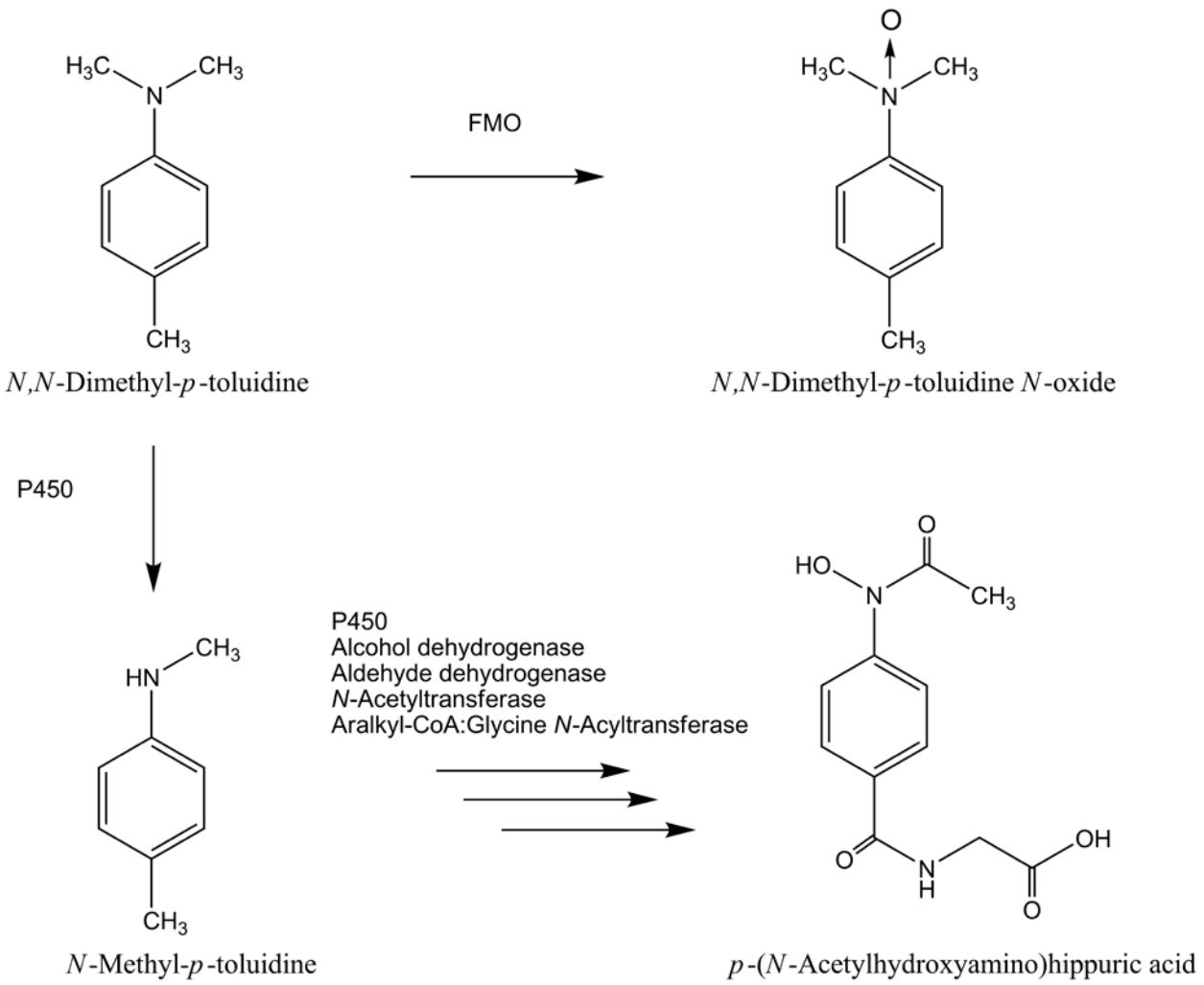


FIGURE 1
Proposed Metabolism of *N,N*-Dimethyl-*p*-toluidine (adapted from Kim *et al*., 2007)
FMO = flavin-containing monooxygenase; P450 = cytochrome P450

approximately 2% *N,N*-dimethyl-*p*-toluidine (approximately 6 mg *N,N*-dimethyl-*p*-toluidine/kg body weight; Potter *et al*., 1988). Both children recovered. *N,N*-Dimethyl-*p*-toluidine-induced methemoglobinemia may be the result of its metabolism to *p*-methylphenylhydroxylamine (Potter *et al*., 1988; Kao *et al*., 1997).

Patients with dental prostheses (methyl methacrylate polymerized with *N,N*-dimethyl-*p*-toluidine) have reported burning and soreness of the mouth. Skin patch tests on these patients have shown strong reactivity to *N,N*-dimethyl-*p*-toluidine within 1 month of use of the dentures (Tosti *et al*., 1990).

Allergic responses to *N,N*-dimethyl-*p*-toluidine may contribute to early aseptic loosening of total hip replacements as well as to contact stomatitis and "denture sore mouth" or "burning mouth" syndrome (Haddad *et al*., 1995). Haddad *et al*. (1996) studied 70 patients, 15 with aseptic loosening less than 2 years after total hip replacement, 25 with satisfactory long-term cemented fixation, five with infected loosening, and 25 awaiting hip arthroplasty. Skin patch tests showed seven positive reactions to *N,N*-dimethyl-*p*-toluidine, all of them in patients with early aseptic loosening.

The role of *N,N*-dimethyl-*p*-toluidine in contact hypersensitivity was investigated in 22 patients with "burning

mouth" syndrome (Dutrée-Meulenberg *et al*., 1992). Twenty of the patients wore a complete or partial denture. Positive patch test reactions to *N*,*N*-dimethyl-*p*-toluidine were seen in three cases – all denture wearers. Verschueren and Bruynzeel (1991) and Tosti *et al*. (1990) also cite *N*,*N*-dimethyl-*p*-toluidine allergy in relation to "burning mouth" syndrome. Santosh *et al*. (1999) reported that a dental student who presented with vesiculobullous lesions on the fingertips following contact with dental materials used in prostheses, showed a positive reaction to a common patch test for *N*,*N*-dimethyl-*p*-toluidine.

N,*N*-Dimethyl-*p*-toluidine allergic reactions may be due to *N*,*N*-dimethyl-*p*-toluidine exposures from bone cements and dental prostheses (Kaaber *et al*., 1979; Kaaber, 1990). *N*,*N*-Dimethyl-*p*-toluidine has been identified in bone cements after storage in air or after long-term implantation in patients (Brauer *et al*., 1986; Tosti *et al*., 1990).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

No studies on the reproductive or developmental toxicity of *N*,*N*-dimethyl-*p*-toluidine in experimental animals or humans were found in the literature.

CARCINOGENICITY

Experimental Animals

In a lifetime study in rats, *N*,*N*-dimethyl-*p*-toluidine was administered at approximately 7 mg/day in the diet (approximately 35 mg/kg per day) (Druckrey *et al*., 1954). No treatment-related tumor response was reported.

Humans

There were no human epidemiology studies examining the potential carcinogenic effects of *N*,*N*-dimethyl-*p*-toluidine reported in the literature. The IARC evaluated surgical implants and other foreign bodies for evidence of carcinogenic risks to humans and found that there was inadequate evidence for evaluation of the carcinogenic potential for most dental and hip replacements (IARC, 1999).

GENETIC TOXICITY

There is some debate in the literature about the mutagenicity of *N*,*N*-dimethyl-*p*-toluidine in bacterial test systems. Up to a maximum nontoxic dose of 70 µg/plate, *N*,*N*-dimethyl-*p*-toluidine was reported to be nonmutagenic in *Salmonella typhimurium* strains TA97, TA98, and TA100, either with or without S9 metabolic activation (Taningher *et al*., 1993). However, using the spot test to evaluate a 300 mg/mL solution (10 µL added to plate) of *N*,*N*-dimethyl-*p*-toluidine, Miller *et al.* (1986) were able to induce mutations in TA100 with and without S9, and in TA104 in the presence of S9; no mutation induction was seen in TA98 with or without S9, consistent with the results of the Taningher *et al*. (1993) study.

N,*N*-Dimethyl-*p*-toluidine was reported to be genotoxic in two mammalian cell assays. The compound was reported to induce trifluorothymidine resistance in L5178Y mouse lymphoma $tk^{+/-}$ cells when tested up to 0.044 µL/mL with S9, and up to 0.24 µL/mL without S9 (IARC, 1999).

In an *in vitro* micronucleus test (a measure of numerical or structural chromosomal damage), *N*,*N*-dimethyl-*p*-toluidine demonstrated evidence of both aneugenic and clastogenic activity (inducing both CREST+ and CREST-MN) in Chinese hamster V79 cells over a concentration range of 0.3 to 1.2 mM in the absence of S9, when cells were analyzed 48 hours after compound addition (Taningher *et al*., 1993). However, this micronucleus study exposed cells for approximately 3 to 4 cell cycles, longer than the recommended 1.5 to 2 cell cycles for *in vitro* micronucleus determination (OECD, 2010), and the level of cytotoxicity induced at higher concentrations could not be accurately assessed.

In vivo, Taningher *et al*. (1993) measured DNA fragmentation by the alkaline elution test in liver of BALB/c mice and Sprague-Dawley rats treated with *N*,*N*-dimethyl-*p*-toluidine by oral gavage or intraperitoneal injection. The greatest DNA elution rate, 2.4 times the mean value seen in the control rats, was obtained in rats 6 hours after oral administration of 8 mmol/kg *N*,*N*-dimethyl-*p*-toluidine, but the increase was not significant; 24 hours after treatment, the elution rate returned to control values. Intraperitoneal administration of 8 mmol/kg in the rat showed only a slight increase in elution rate 2 hours after treatment, and the rate returned to control values after 24 hours. DNA fragmentation in mouse liver was not increased 2 hours after intraperitoneal administration of 2 mmol/kg *N*,*N*-dimethyl-*p*-toluidine (highest dose tolerated by mice), but a marginal response was seen 24 hours after intraperitoneal administration of 1 mmol/kg ($P<0.05$).

STUDY RATIONALE

N,N-Dimethyl-*p*-toluidine was nominated for toxicology and carcinogenesis studies by the National Cancer Institute based on the potential for human exposure through its use in dental materials and bone cements, and the lack of toxicity and carcinogenicity data.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

N,N-Dimethyl-*p*-toluidine

N,N-Dimethyl-*p*-toluidine was obtained from Alfa Aesar, a Johnson Matthey Company (Ward Hill, MA), in two lots (H3124A and J7601A). Lot H3124A was used in the 3-month studies. The remainder of lot H3124A was combined with lot J7601A to make lot 050404 which was used in the 2-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory at Battelle's Chemistry Support Services (Columbus, Ohio) and by the study laboratory at Battelle Columbus Operations (Columbus, OH), and Karl Fischer titration and elemental analyses were performed by Galbraith Laboratories, Inc. (Knoxville, TN), and by Prevalere Life Sciences, Inc. (Whitesboro, NY) (Appendix I). Reports on analyses performed in support of the *N,N*-dimethyl-*p*-toluidine studies are on file at the National Institute of Environmental Health Sciences.

All lots of the chemical, a pale-yellow liquid, were identified as *N,N*-dimethyl-*p*-toluidine by infrared spectroscopy. Identity confirmation of lot H3124A and combined lot 050404 was conducted by proton and carbon-13 nuclear magnetic resonance spectroscopy.

For lot H3124A, Karl Fischer titration indicated approximately 0.22% water. Boiling point determination and elemental analyses results for carbon, hydrogen, and nitrogen were consistent with theoretical values. Gas chromatography with flame ionization detection (GC/FID) indicated one major peak and two impurities (0.1% and 0.2%) with peak areas greater than or equal to 0.1% of the major peak area. Differential scanning calorimetry indicated a purity of 99.8%. The overall purity of lot H3124A was determined to be greater than 99%.

For lot J7601A, GC/FID indicated one major peak and three impurities (0.1%, 0.1%, and 0.2%) with peak areas greater than or equal to 0.1% of the total peak area. The overall purity of lot J7601A was determined to be greater than 99% and was sufficiently similar to lot H3124A to allow the two lots to be combined.

For combined lot 050404, Karl Fischer titration indicated approximately 0.2% water; elemental analyses for carbon, hydrogen, and nitrogen were consistent with theoretical values. GC/FID indicated one major peak and four impurities (0.2%, 0.1%, 0.2%, and 0.1%) with peak areas greater than or equal to 0.1% of the total peak area. The overall purity of combined lot 050404 was determined to be greater than 99%.

To ensure stability, the bulk chemical was stored in amber glass containers sealed with Teflon®-lined lids at room temperature. Periodic reanalyses of the bulk chemical using GC/FID were performed at the beginning, middle, and end of the 3-month studies and at least every 6 months during the 2-year studies; no degradation of the chemical was observed.

Corn Oil

USP-grade corn oil was obtained in multiple lots from Spectrum Chemicals and Laboratory Products (Gardena, CA). Periodic analyses of the corn oil vehicle using potentiometric titration demonstrated peroxide concentrations below the acceptable limit of 3 mEq/kg.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

Dose formulations were prepared by adding the appropriate amount of *N,N*-dimethyl-*p*-toluidine to corn oil to achieve the desired concentration (Table I1). Dose formulations were prepared three times for the 3-month studies and approximately monthly for the 2-year studies.

The 400 mg/mL dose formulation was prepared and observed to be a true solution, therefore, no homogeneity or gavageability studies were performed. Stability studies of a 1.0 mg/mL formulation in corn oil were performed using GC/FID. Stability was confirmed for up to 44 days for formulations stored in amber glass containers sealed with Teflon®-lined lids, protected from light, at up to room temperature and for at least 3 hours under simulated animal room conditions.

Periodic analyses of the dose formulations were conducted by the study laboratory using GC/FID. During

the 3-month studies, the dose formulations were analyzed at the beginning, midpoint, and end of the studies; animal room samples of these dose formulations were also analyzed (Table I2). Of the dose formulations analyzed and used, all 13 for rats and all 15 for mice were within 10% of the target concentrations; all 13 animal room samples for rats and all 15 for mice were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed at least every 3 months; animal room samples were also analyzed (Table I3). Of the dose formulations analyzed and used, all 30 for rats and all 30 for mice were within 10% of the target concentrations; all 12 animal room samples for rats and all 12 for mice were within 10% of the target concentrations.

3-MONTH STUDIES

The 3-month studies were conducted to evaluate the cumulative toxic effects of repeated exposure to *N,N*-dimethyl-*p*-toluidine and to determine the appropriate doses to be used in the 2-year studies. The *N,N*-dimethyl-*p*-toluidine oral LD_{50} value in rats is 1,650 mg/kg. The oral TD_{Lo} value in mice is 250 mg/kg (RTECS, 2011). The doses for the *N,N*-dimethyl-*p*-toluidine 3-month studies were selected based on these LD_{50} values to deliver 0, 62.5, 125, 250, 500, and 1,000 mg/kg in rats, and 0, 15, 30, 60, 125, and 250 mg/kg in mice. The low dose was approximately 10 times the amount ingested in children that was reported to cause methemoglobinemia (Potter *et al*., 1988; Kao *et al*., 1997). The chemical was administered by oral gavage because the chemical was not palatable by the feed route (Fomby and Graves, 2001).

Male and female F344/N rats and B6C3F1/N mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats and mice were 4 to 5 weeks old. Animals were quarantined for 11 (male rats), 12 (female rats), 14 (male mice), or 13 (female mice) days and were 5 to 7 weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. At 4 weeks and the end of the studies, serologic analyses were performed on five male and five female sentinel rats and mice using the protocols of the NTP Sentinel Animal Program (Appendix K).

Groups of 10 male and 10 female rats were administered *N,N*-dimethyl-*p*-toluidine in corn oil by gavage at doses of 62.5, 125, 250, 500, or 1,000 mg/kg body weight, 5 days per week for 14 weeks. Additional clinical pathology groups of 10 male and 10 female rats received the same doses for 25 days. Groups of 10 male and 10 female mice received *N,N*-dimethyl-*p*-toluidine in corn oil by gavage at doses of 15, 30, 60, 125, or 250 mg/kg, 5 days per week for 14 weeks. Vehicle control animals received the corn oil vehicle alone. Feed and water were available *ad libitum*. Rats and female mice were housed five per cage and male mice were housed individually. The animals were weighed and clinical findings were recorded at study start, on the first Friday after dosing started, weekly thereafter, and at study termination. Details of the study design and animal maintenance are summarized in Table 1.

Blood was collected for hematology and clinical chemistry analyses from clinical pathology rats on day 25 and from surviving core study rats at study termination; on day 88, blood was collected from core study rats for hemoglobin and methemoglobin only. Blood was collected for hematology analyses from surviving mice at study termination. Blood for methemoglobin analyses was collected within 4 hours of dosing after 5 consecutive days of dosing; otherwise, blood was collected within 24 hours of dosing. At all timepoints, the animals were anesthetized with a CO_2/O_2 mixture and blood was collected from the retroorbital sinus. Blood for hematology analyses was placed in tubes containing EDTA as the anticoagulant. Erythrocyte, platelet, leukocyte (total and differential), and reticulocyte counts; automated hematocrit values; hemoglobin concentration; mean cell volume; mean cell hemoglobin; and mean cell hemoglobin concentration were determined using an ADVIA 120 analyzer (Bayer Diagnostics Division, Tarrytown, NY). Blood smears for rats and mice were stained with a Romanowsky-type stain and evaluated microscopically for blood morphology and enumeration of nucleated erythrocytes/100 white cells. Blood smears, prepared from supravitally-stained whole blood, were used for the enumeration of Heinz bodies (i.e., as a Heinz body:erythrocyte ratio using the Miller disc method) (Brecher and Schneiderman, 1950). Methemoglobin concentration was determined using the method of Evelyn and Malloy (1938) and reagents purchased from Sigma Chemical Company (St. Louis, MO). Blood samples for clinical chemistry analyses were placed in tubes containing separator gel and allowed to clot. After clot retraction occurred, the samples were centrifuged, and the serum was aliquoted for assay of serum chemistry analytes using a Hitachi 911 analyzer (Boehringer Mannheim, Indianapolis, IN). The parameters measured are listed in Table 1.

At the end of the 3-month studies, samples were collected for sperm motility and vaginal cytology evaluations on core study rats administered 0, 62.5, 125, or 250 mg/kg and mice administered 0, 15, 30, or 60 mg/kg. The parameters evaluated are listed in

Table 1. For 12 consecutive days prior to scheduled terminal kill, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (except eyes were first fixed in Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on vehicle control, 500, and 1,000 mg/kg core study rats and vehicle control, 125, and 250 mg/kg mice. The bone marrow, kidney, liver, lung, nose, spleen, and thymus of rats and mice; the forestomach and mesenteric lymph node of rats; the heart and glandular stomach of female rats; and the mandibular and mesenteric lymph nodes and trachea of mice were examined in the remaining dosed groups. Table 1 lists the tissues and organs routinely examined.

After a review of the laboratory reports and selected histopathology slides by a quality assessment pathologist, the findings and reviewed slides were submitted to a NTP Pathology Working Group (PWG) coordinator for a second independent review. Any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists were resolved by the NTP pathology peer review process. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, NTP pathologist, reviewing pathologist(s), and the PWG coordinator. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

2-YEAR STUDIES

Study Design

Groups of 50 male and 50 female rats and mice were administered *N,N*-dimethyl-*p*-toluidine in corn oil by gavage at doses of 6, 20, or 60 mg/kg 5 days per week for 104 (male rats) or 105 weeks. Vehicle control animals received the corn oil vehicle alone. Additional clinical pathology groups of 10 male and 10 female rats were administered the same doses for 86 days. Formulations were administered at a volume of 2.5 mL/kg (rats) or 5 mL/kg (mice) and were calculated based on each animal's most recent body weight.

Source and Specification of Animals

Male and female F344/N rats and B6C3F1/N mice were obtained from Taconic Farms, Inc. (Germantown, NY), for use in the 2-year studies. Animals were quarantined for 13 (male rats), 14 (female rats), 12 (male mice), or 11 (female mice) days before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats and mice were 5 to 7 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix K).

Animal Maintenance

All animal studies were conducted in an animal facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International. Studies were approved by the Battelle Columbus Operations Animal Care and Use Committee and conducted in accordance with all relevant NTP animal care and use policies and applicable federal, state, and local regulations and guidelines. Male rats were housed up to three per cage, female rats were housed five per cage, female mice were housed three to five per cage, and male mice were housed individually. Feed and water were available *ad libitum*. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix J.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded every 4 weeks beginning with week 5 and at the end of the studies. Body weights were recorded on day 1, weekly for the first 13 weeks, every 4 weeks thereafter, and at terminal kill.

After anesthetization with a CO_2/O_2 mixture, blood was taken from the retroorbital sinus of clinical pathology rats on day 86 for hematology analyses. Blood was placed in tubes containing EDTA as the anticoagulant. Hematology analyses were performed as described for the 3-month studies. The parameters measured are listed in Table 1.

Complete necropsies and microscopic examinations were performed on all core study rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin (except eyes were first fixed in Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. Tissues examined microscopically are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The report, slides, paraffin blocks, residual wet tissues, and pathology data were sent to the NTP Archives for inventory, slide/block match, wet tissue audit, and storage. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a quality assessment pathologist evaluated slides from all tumors and all potential target organs, which included the liver and nose of rats and mice; the bone marrow, kidney, and spleen of rats; and the lung of mice.

The quality assessment report and the reviewed slides were submitted to the NTP PWG coordinator, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the coordinator to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell *et al.* (1986).

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of *N,N*-Dimethyl-*p*-toluidine

3-Month Studies	2-Year Studies
Study Laboratory	
Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)
Strain and Species	
F344/N rats B6C3F1/N mice	F344/N rats B6C3F1/N mice
Animal Source	
Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies	
Rats: 11 (males) or 12 (females) days Mice: 14 (males) or 13 (females) days	Rats: 13 (males) or 14 (females) days Mice: 12 (males) or 11 (females) days
Average Age When Studies Began	
Rats: 5 to 6 weeks Mice: 6 to 7 weeks	Rats: 6 to 7 weeks Mice: 5 to 6 weeks
Date of First Dose	
Rats: October 20 (males) or 21 (females), 2003 Mice: October 23 (males) or 22 (females), 2003	Rats: October 20 (males) or 21 (females), 2004 Mice: October 26 (males) or 25 (females), 2004
Duration of Dosing	
5 days/week for 14 weeks	5 days/week for 104 (male rats) or 105 weeks
Date of Last Dose	
Rats: January 19 (males) or 20 (females), 2004 Mice: January 23 (males) or 22 (females), 2004	Rats: October 17 (males) or 19 (females), 2006 Mice: October 26 (males) or 24 (females), 2006
Necropsy Dates	
Rats: January 20 (males) or 21 (females), 2004 Mice: January 23 (males) or 22 (females), 2004	Rats: October 16-18 (males) or 18-20 (females), 2006 Mice: October 25-27 (males) or 23-25 (females), 2006
Average Age at Necropsy	
19 to 20 weeks	110 to 111 weeks
Size of Study Groups	
Core study: 10 male and 10 female rats and mice Clinical pathology study: 10 male and 10 female rats	Core study: 50 male and 50 female rats and mice Clinical pathology study: 10 male and 10 female rats
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 3-month studies
Animals per Cage	
Rats: 5 Mice: 1 (males) or 5 (females)	Rats: 1 to 3 (males) or 5 (females) Mice: 1 (males) or 3 to 5 (females)
Method of Animal Identification	
Tail tattoo	Tail tattoo
Diet	
Irradiated NTP-2000 wafer feed (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed weekly	Same as 3-month studies

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of *N,N*-Dimethyl-*p*-toluidine

3-Month Studies	2-Year Studies
Water Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*	Same as 3-month studies
Cages Polycarbonate (Lab Products, Inc., Seaford, DE), changed weekly (male mice) or twice weekly	Same as 3-month studies
Bedding Irradiated Sani-Chips (P.J. Murphy Forest Products Corp., Montville, NY), changed weekly (male mice) or twice weekly	Same as 3-month studies
Cage Filters Spun-bonded polyester (Snow Filtration Co., Cincinnati, OH), changed every 2 weeks	Same as 3-month studies
Racks Stainless steel (Lab Products, Seaford, DE), changed and rotated every 2 weeks	Same as 3-month studies
Animal Room Environment Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Doses Rats: 0, 62.5, 125, 250, 500, or 1,000 mg/kg in corn oil vehicle (dosing volume 2.5 mL/kg) Mice: 0, 15, 30, 60, 125, or 250 mg/kg in corn oil vehicle (dosing volume 5 mL/kg)	0, 6, 20, or 60 mg/kg in corn oil vehicle (dosing volume=2.5 mL/kg for rats or 5 mL/kg for mice)
Type and Frequency of Observation Observed twice daily; animals were weighed and clinical findings were recorded initially, on the first Friday after dosing started, weekly thereafter, and at the end of the studies.	Observed twice daily; core study animals were weighed initially, weekly for the first 13 weeks, every 4 weeks thereafter, and at the end of the studies; clinical findings were recorded every 4 weeks beginning with week 5 and at the end of the studies.
Method of Kill Carbon dioxide asphyxiation	Same as 3-month studies
Necropsy Necropsies were performed on all core study animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all core study animals.
Clinical Pathology Blood was collected from the retroorbital sinus of clinical pathology rats on day 25, from core study rats on day 88 (hemoglobin and methemoglobin), and from core study rats at the end of the study for hematology and clinical chemistry. Blood was collected from the retroorbital sinus of mice at the end of the study for hematology. ***Hematology:*** hematocrit; hemoglobin and methemoglobin concentrations; erythrocyte, reticulocyte, nucleated erythrocyte, platelet, and Heinz body counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatinine kinase, sorbitol dehydrogenase, and bile acids	Blood was collected from the retroorbital sinus of clinical pathology rats on day 86 for hematology. ***Hematology:*** hematocrit; hemoglobin and methemoglobin concentrations; erythrocyte, reticulocyte, platelet, and Heinz body counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of *N*,*N*-Dimethyl-*p*-toluidine

3-Month Studies	2-Year Studies
Histopathology Histopathology was performed on vehicle control, 500, and 1,000 mg/kg core study rats and vehicle control, 125, and 250 mg/kg mice. In addition to gross lesions and tissue masses, the following tissues were examined to a no-effect level: adrenal gland, bone (including marrow), brain, clitoral gland, esophagus, gallbladder (mice), heart (including aorta), large intestine (cecum, colon, and rectum), small intestine (duodenum, jejunum, and ileum), kidney, liver, lung (and mainstem bronchi), lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus. In addition, the bone marrow, kidney, liver, lung, nose, spleen, and thymus of rats and mice; the forestomach and mesenteric lymph node of rats; the heart and glandular stomach of female rats; and the mandibular and mesenteric lymph nodes and trachea of mice were examined in the remaining groups.	Complete histopathology was performed on all core study animals. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone (including marrow), brain, clitoral gland, esophagus, eye, gallbladder (mice), Harderian gland, heart (including aorta), large intestine (cecum, colon, and rectum), small intestine (duodenum, jejunum, and ileum), kidney, liver, lung (and mainstem bronchi), lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, uterus, and Zymbal's gland (male rats).
Sperm Motility and Vaginal Cytology At the end of the studies, spermatid and sperm samples were collected from male rats in the vehicle control, 62.5, 125, and 250 mg/kg groups and from male mice in the vehicle control, 15, 30, and 60 mg/kg groups. The following parameters were evaluated: spermatid heads per testis and per gram testis, sperm motility, and sperm per cauda epididymis and per gram cauda epididymis. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from female rats administered 0, 62.5, 125, or 250 mg/kg and from female mice administered 0, 15, 30, or 60 mg/kg.	

STATISTICAL METHODS

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals found dead of other than natural causes were censored; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A4, B1, B4, C1, C4, D1, and D4 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A2, B2, C2, and D2) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., mesentery, pleura, peripheral nerve, skeletal muscle, tongue, tooth, and Zymbal's gland) before microscopic evaluation, the denominators consist of the number of animals that had a gross abnormality. When neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A2, B2, C2, and D2 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal kill.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion at that site or if it survived until terminal kill; if the animal died prior to terminal kill and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time (Bailer and Portier, 1988). Unless otherwise specified, a value of k=3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier (1988) following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344 rats and B6C3F1 mice (Portier *et al.*, 1986). Bailer and Portier (1988) showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams (1993).

Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-related trend. Continuity-corrected Poly-3 tests were used in the analysis of lesion incidence, and reported P values are one sided. The significance of lower incidences or decreasing trends in lesions is represented as 1–P with the letter N added (e.g., P=0.99 is presented as P=0.01N).

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis. Proportions of regular cycling females in each dosed group were compared to the control group using

the Fisher exact test (Gart *et al.*, 1979). Tests for extended periods of estrus, diestrus, metestrus, and proestrus, as well as skipped estrus and skipped diestrus, were constructed based on a Markov chain model proposed by Girard and Sager (1987). For each dose group, a transition probability matrix was estimated for transitions among the proestrus, estrus, metestrus, and diestrus stages, with provision for extended stays within each stage as well as for skipping estrus or diestrus within a cycle. Equality of transition matrices among dose groups and between the control group and each dosed group was tested using chi-square statistics.

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical database must be generally similar. One significant factor affecting the background incidence of neoplasms at a variety of sites is diet. In 1995, the NTP incorporated a new diet (NTP-2000) that contains less protein and more fiber and fat than the NIH-07 diet previously used in toxicity and carcinogenicity studies (Rao, 1996, 1997). The NTP historical database contains all studies that use the NTP-2000 diet with histopathology findings completed within the most recent 5-year period. A second potential source of variability is route of administration. In general, the historical database for a given study will include studies using the same route of administration, and the overall incidences of neoplasms for all routes of administration are included for comparison, including the present study.

QUALITY ASSURANCE METHODS

The 3-month and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assurance contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICOLOGY

The genetic toxicity of *N,N*-dimethyl-*p*-toluidine was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and *Escherichia coli*, increases in the frequency of micronucleated erythrocytes in mouse peripheral blood, and DNA damage in mice and Sprague-Dawley rats. Micronuclei (literally "small nuclei" or Howell-Jolly bodies) are biomarkers of induced structural or numerical chromosomal alterations and are formed when acentric fragments or whole chromosomes fail to incorporate into either of two daughter nuclei during cell division (Schmid, 1975; Heddle *et al.*, 1983). The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies have evolved from an earlier effort by the NTP to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term *in vitro* and *in vivo* genetic toxicity tests (structure-activity relationships). The short-term tests were originally developed to clarify proposed mechanisms of chemical-induced DNA damage based on the relationship between electrophilicity and mutagenicity (Miller and Miller, 1977) and the somatic mutation theory of cancer (Straus, 1981; Crawford, 1985). However, it should be noted that not all cancers arise through genotoxic mechanisms.

DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites (Ashby and Tennant, 1991). A positive response in the *Salmonella* test was shown to be the most predictive *in vitro* indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens) (Tennant *et al.*, 1987; Zeiger *et al.*, 1990). Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. However, these other tests can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

The predictivity for carcinogenicity of a positive response in acute *in vivo* bone marrow chromosome aberration or micronucleus tests appears to be less than that in the *Salmonella* test (Shelby *et al.*, 1993; Shelby and Witt, 1995). However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity; a weak response in one sex only or negative results in both sexes in this

assay do not correlate well with either negative or positive results in rodent carcinogenicity studies (Witt *et al.*, 2000). The comet assay detects a variety of DNA damage including single and double strand breaks, DNA-DNA and DNA-protein crosslinks, and alkali labile sites in individual cells (Collins *et al*., 2008). A comprehensive study that assessed the correlation between positive comet assay data in a variety of target tissues of rats and mice and rodent carcinogenicity concluded that a positive comet assay response in at least one organ of one species is well correlated with rodent carcinogenicity (Sasaki *et al*., 2000). An in-depth examination of the relationship between comet assay results and rodent carcinogenicity is currently underway by the NTP. Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of *in vivo* genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical.

RESULTS

RATS

3-MONTH STUDY

All 1,000 mg/kg male and female rats and one 500 mg/kg male rat died by study day 3 (Table 2). The final mean body weights and mean body weight gains of all surviving dosed groups of males and females were significantly less than those of the vehicle controls (Table 2 and Figure 2). Clinical findings associated with exposure to *N*,*N*-dimethyl-*p*-toluidine included cyanosis, abnormal breathing, and lethargy in groups administered 250 mg/kg or greater.

TABLE 2
Survival and Body Weights of Rats in the 3-Month Gavage Study of *N*,*N*-Dimethyl-*p*-toluidine[a]

Dose (mg/kg)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	99 ± 3	327 ± 2	228 ± 4	
62.5	10/10	99 ± 3	296 ± 5**	197 ± 5**	90
125	10/10	98 ± 3	289 ± 8**	191 ± 8**	88
250	10/10	97 ± 2	252 ± 5**	155 ± 4**	77
500	9/10[c]	99 ± 3	234 ± 9**	135 ± 8**	72
1,000	0/10[c]	99 ± 3	—	—	—
Female					
0	10/10	93 ± 3	193 ± 3	100 ± 2	
62.5	10/10	92 ± 3	183 ± 3*	91 ± 2*	95
125	10/10	92 ± 3	172 ± 4**	80 ± 2**	89
250	10/10	93 ± 3	174 ± 3**	80 ± 3**	90
500	10/10	92 ± 2	175 ± 3**	82 ± 2**	91
1,000	0/10[c]	92 ± 2	—	—	—

* Significantly different (P≤0.05) from the vehicle control group by Williams' test
** P≤0.01
[a] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[b] Number of animals surviving at 14 weeks/number initially in group
[c] Week of deaths: 1

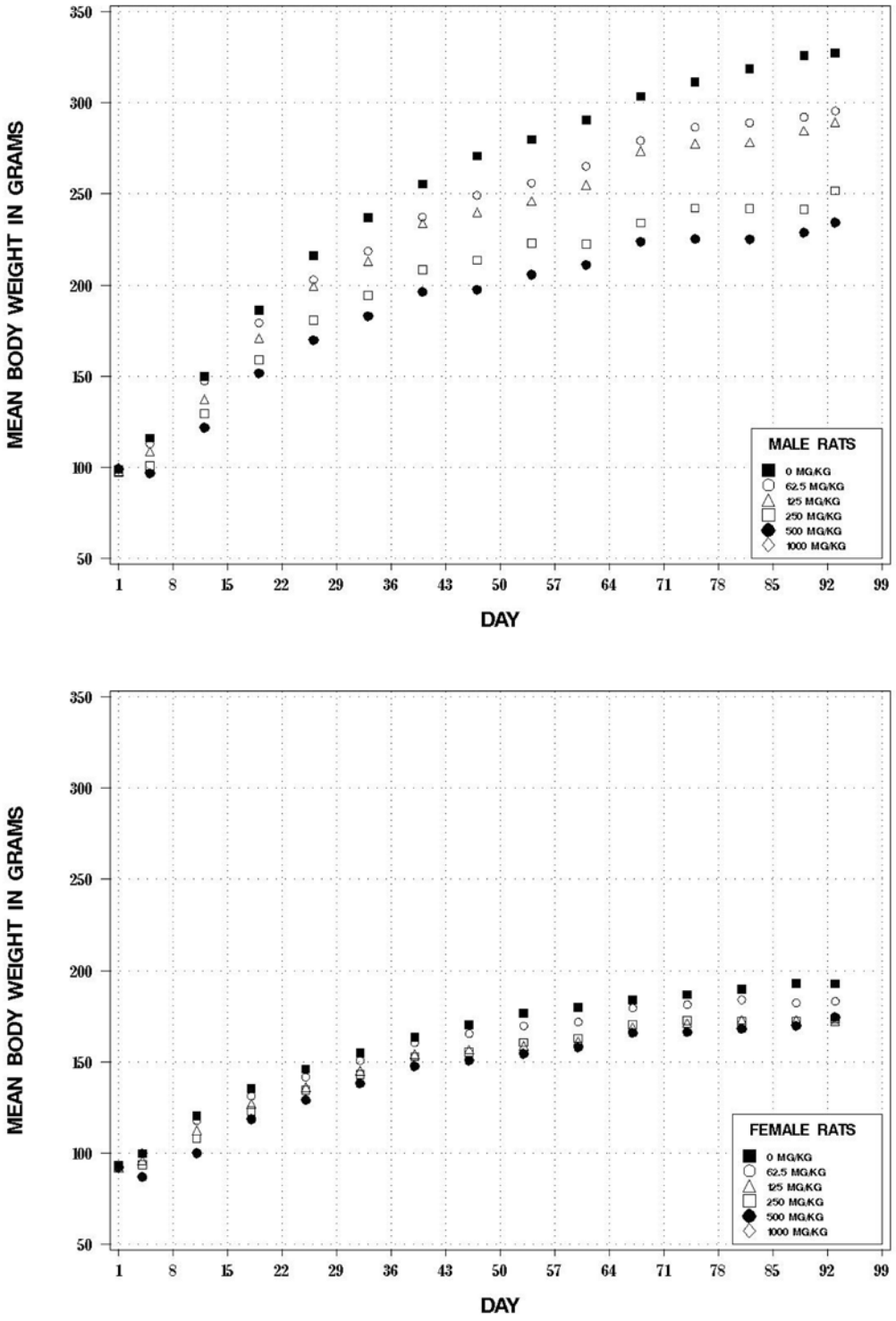


FIGURE 2
Growth Curves for Rats Administered *N,N*-Dimethyl-*p*-toluidine by Gavage for 3 Months

The hematology data for rats are presented in Tables 3 and F1. The hematology findings were consistent with methemoglobinemia and Heinz body formation (Plates 1 and 2) resulting in a macrocytic, hypochromic, responsive anemia. In general, these changes were dose-related, occurred at both timepoints evaluated, and involved all dosed groups of both sexes. The methemoglobinemia was described by a considerable treatment-related increase in methemoglobin values. The anemia was characterized by dose-related decreases in the erythron including decreases in hematocrit values, hemoglobin concentrations, and erythrocyte counts. The greatest magnitudes of decrease occurred in the 500 mg/kg groups on day 25; the decrease was greater than 20% for hematocrit and hemoglobin values and close to 40% for erythrocyte counts. By week 14, there was some amelioration in the severities of the anemia. Erythrocyte macrocytosis was characterized by increases in mean cell volume and mean cell hemoglobin values indicating that the circulating erythrocytes were larger than those of the concurrent vehicle controls. Erythrocyte hypochromia was evidenced by decreases in mean cell hemoglobin concentration values, indicating that the circulating erythrocytes did not have the normal intracellular hemoglobin content. An erythropoietic response to the anemia was characterized by substantially increased reticulocyte and nucleated erythrocyte counts. Decreases in leukocyte counts occurred in 250 and 500 mg/kg male and female rats on day 25. Decreases in lymphocyte counts mimicked the leukocyte count decreases; these changes were consistent with physiologic responses to stress.

On day 25, markers of hepatocellular injury, serum activities of alanine aminotransferase and sorbitol dehydrogenase, demonstrated dose-related increases in essentially all dosed groups of males and females; the 500 mg/kg animals had increases of greater than threefold (Table F1). By week 14, the increases in alanine aminotransferase and sorbitol dehydrogenase activities had ameliorated or resolved in all dosed groups. Serum concentrations of total bile acids, a marker of hepatic function/injury and cholestasis, were increased in higher-dose animals; the 500 mg/kg groups were the most consistently affected demonstrating a greater than threefold increase at both timepoints. Another marker of cholestasis (alkaline phosphatase activity), however, demonstrated decreases (day 25) or no change (week 14). Thus, it would appear that the increases in bile acid concentrations were not related to a cholestatic event. Serum albumin concentrations (and by extension, serum total protein) were increased in essentially all dosed male and female groups at both timepoints. The increases in albumin and total protein were proportional, suggesting that the increases were related to a physiologic hemoconcentration-type response (i.e., dehydration). This supposition was supported by the substantially lower body weights suggesting that the treated animals did not eat and, therefore, drink as expected.

The absolute and relative liver weights of all surviving dosed groups of males and females were significantly greater than those of the vehicle controls (Table G1). The absolute right kidney weights of 125 and 500 mg/kg males and all surviving dosed groups of females and the relative right kidney weights of all surviving dosed groups of males and females were significantly greater than those of the vehicle controls. The absolute right testis weight of 500 mg/kg males was significantly less than that of the vehicle controls, but the relative right testis weights of all surviving groups were significantly greater than that of the vehicle controls.

There was a dose-related decrease in the proportion of cycling females, with only four females in the 250 mg/kg group having regular cycles (Table H2), despite a loss of cells due to technical errors that compromised the evaluation of estrous cyclicity. Females in the 125 and 250 mg/kg groups spent a significantly higher proportion of time in extended diestrus compared to the vehicle control group (P=0.0022 and P=0.0002, respectively). There were no significant differences in spermatid or epididymal spermatozoal measurements of male rats administered 62.5, 125, or 250 mg/kg *N,N*-dimethyl-*p*-toluidine when compared to the vehicle control group; however, there were significant decreases in left cauda epididymis and left epididymis weights in the 250 mg/kg group (Table H1). Based on these results, *N,N*-dimethyl-*p*-toluidine has the potential to be a reproductive toxicant in female rats but not in male rats.

Many of the histological findings in 1,000 mg/kg males and females were not observed in the lower dose groups and were considered to be related to stress and/or nonspecific toxicity. These lesions included centrilobular hepatocellular necrosis and fatty change of the liver, ulceration of the forestomach, renal tubule dilatation, red pulp atrophy of the spleen, and necrosis and hemorrhage of the thymus. Centrilobular hepatocyte necrosis was characterized by necrosis of individual hepatocytes in the centrilobular regions. The individual necrotic hepatocytes presented with finely granular chromatin fragments outlining the nuclear region and with a loss of cytoplasmic detail without condensation. Centrilobular fatty change resulted in clear, sharply defined, intracytoplasmic vacuoles primarily within centrilobular hepatocytes. Ulceration of the forestomach was diagnosed when there was full-thickness necrosis of the squamous epithelium, and included lesions in which a focus of

TABLE 3
Selected Hematology Data for Rats in the 3-Month Gavage Study of *N,N*-Dimethyl-*p*-toluidine[a]

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg
Male					
n					
Day 25	10	10	9	10	8
Day 88	10	10	10	10	9
Week 14	10	10	10	10	9
Hematocrit (%)					
Day 25	49.7 ± 0.3	45.0 ± 0.5**	42.8 ± 0.4**	40.2 ± 0.5**	39.2 ± 0.5**
Week 14	46.1 ± 0.4	42.1 ± 0.5**	42.3 ± 0.4**	42.1 ± 0.4**	42.4 ± 0.7**
Hemoglobin (g/dL)					
Day 25	15.3 ± 0.1	13.3 ± 0.1**	12.5 ± 0.1**	11.8 ± 0.1**	11.0 ± 0.1**
Week 14	14.8 ± 0.1	13.0 ± 0.2**	13.0 ± 0.1**	12.9 ± 0.1**	12.7 ± 0.2**
Erythrocytes (10^6/µL)					
Day 25	8.26 ± 0.05	7.44 ± 0.07**	6.79 ± 0.07**	5.97 ± 0.09**	5.06 ± 0.05**
Week 14	8.62 ± 0.07	7.43 ± 0.08**	6.94 ± 0.05**	6.40 ± 0.07**	6.19 ± 0.07**
Reticulocytes (10^6/µL)					
Day 25	0.26 ± 0.01	0.50 ± 0.01**	0.64 ± 0.01**	0.94 ± 0.03**	1.08 ± 0.03**
Week 14	0.25 ± 0.01	0.50 ± 0.01**	0.60 ± 0.02**	0.76 ± 0.01**	0.89 ± 0.04**
Nucleated erythrocytes/100 leukocytes					
Day 25	0.2 ± 0.1	1.3 ± 0.4*	1.3 ± 0.5*	4.7 ± 0.7**	21.6 ± 2.1**
Week 14	0.2 ± 0.1	0.9 ± 0.2*	2.0 ± 0.4**	1.7 ± 0.3**	3.6 ± 0.6**
Mean cell volume (fL)					
Day 25	60.2 ± 0.2	60.5 ± 0.2	63.1 ± 0.2**	67.5 ± 0.6**	77.5 ± 0.5**
Week 14	53.5 ± 0.3	56.6 ± 0.3**	61.1 ± 0.3**	65.8 ± 0.3**	68.5 ± 0.6**
Mean cell hemoglobin (pg)					
Day 25	18.5 ± 0.1	17.9 ± 0.1	18.4 ± 0.1	19.7 ± 0.1**	21.8 ± 0.1**
Week 14	17.2 ± 0.1	17.5 ± 0.1*	18.7 ± 0.1**	20.1 ± 0.1**	20.6 ± 0.2**
Mean cell hemoglobin concentration (g/dL)					
Day 25	30.8 ± 0.1	29.7 ± 0.1**	29.2 ± 0.2**	29.2 ± 0.1**	28.2 ± 0.1**
Week 14	32.1 ± 0.1	31.0 ± 0.2**	30.7 ± 0.1**	30.5 ± 0.1**	30.0 ± 0.1**
Methemoglobin (g/dL)					
Day 25	0.35 ± 0.03	0.90 ± 0.04**	1.56 ± 0.04**[b]	1.95 ± 0.05**	1.63 ± 0.06**
Day 88	0.38 ± 0.02	1.37 ± 0.08**	1.95 ± 0.07**	2.29 ± 0.08**	2.03 ± 0.08**
Methemoglobin (% hemoglobin)					
Day 25	2.40 ± 0.22	6.70 ± 0.30**	12.44 ± 0.41**	16.60 ± 0.31**	14.75 ± 0.56**
Day 88	2.44 ± 0.18[c]	10.10 ± 0.55**	15.50 ± 0.48**	18.20 ± 0.53**	17.67 ± 0.71**
Heinz bodies (% erythrocytes)					
Day 25	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.6**	14.5 ± 1.9**	23.5 ± 2.6**
Week 14	0.0 ± 0.0	0.5 ± 0.2**	2.8 ± 0.3**	4.1 ± 0.4**	2.9 ± 0.8**

TABLE 3
Selected Hematology Data for Rats in the 3-Month Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg
Female					
n					
Day 25	10	10	10	10	10
Day 88	9	10	10	10	10
Week 14	10	10	9	10	10
Hematocrit (%)					
Day 25	48.8 ± 0.4	44.9 ± 0.3**	43.4 ± 0.6**	40.8 ± 0.5**	37.0 ± 0.5**
Week 14	45.2 ± 0.5	41.3 ± 0.5**	40.0 ± 0.6**	39.0 ± 0.4**	40.7 ± 0.3**
Hemoglobin (g/dL)					
Day 25	15.1 ± 0.1	13.3 ± 0.1**	12.8 ± 0.2**	11.7 ± 0.1**	10.8 ± 0.2**
Week 14	14.8 ± 0.1	12.8 ± 0.1**	12.7 ± 0.1**	12.0 ± 0.2**	12.4 ± 0.1**
Erythrocytes (10^6/μL)					
Day 25	8.36 ± 0.07	7.42 ± 0.07**	6.90 ± 0.10**	5.93 ± 0.05**	5.15 ± 0.08**
Week 14	8.16 ± 0.07	6.84 ± 0.08**	6.59 ± 0.10**	6.08 ± 0.10**	5.72 ± 0.06**
Reticulocytes (10^6/μL)					
Day 25	0.18 ± 0.01	0.55 ± 0.02**	0.62 ± 0.03**	0.99 ± 0.05**	1.07 ± 0.04**
Week 14	0.26 ± 0.01	0.50 ± 0.03**	0.54 ± 0.02**	0.90 ± 0.02**	1.11 ± 0.04**
Nucleated erythrocytes/100 leukocytes					
Day 25	0.4 ± 0.2	1.6 ± 0.3**	3.2 ± 0.4**	4.1 ± 0.6**	16.8 ± 1.5**
Week 14	0.7 ± 0.3	1.4 ± 0.3	2.2 ± 0.3**	3.7 ± 0.4**	5.8 ± 0.7**
Mean cell volume (fL)					
Day 25	58.4 ± 0.1	60.5 ± 0.2**	62.9 ± 0.3**	68.7 ± 0.4**	71.9 ± 0.6**
Week 14	55.4 ± 0.2	60.4 ± 0.2**	60.7 ± 0.4**	64.2 ± 0.5**	71.2 ± 0.5**
Mean cell hemoglobin (pg)					
Day 25	18.0 ± 0.1	17.9 ± 0.1	18.5 ± 0.1**	19.8 ± 0.1**	20.9 ± 0.1**
Week 14	18.1 ± 0.0	18.7 ± 0.1**	19.3 ± 0.2**	19.8 ± 0.1**	21.7 ± 0.1**
Mean cell hemoglobin concentration (g/dL)					
Day 25	30.9 ± 0.1	29.5 ± 0.1**	29.4 ± 0.1**	28.8 ± 0.1**	29.0 ± 0.2**
Week 14	32.7 ± 0.1	31.1 ± 0.1**	31.9 ± 0.2**	30.9 ± 0.2**	30.5 ± 0.1**
Methemoglobin (g/dL)					
Day 25	0.37 ± 0.02	0.86 ± 0.07**	1.63 ± 0.05**	1.86 ± 0.05**	1.65 ± 0.03**
Day 88	0.38 ± 0.01	1.49 ± 0.07**	2.20 ± 0.13**	2.49 ± 0.10**	1.75 ± 0.07**
Methemoglobin (% hemoglobin)					
Day 25	2.70 ± 0.15	6.40 ± 0.58**	12.80 ± 0.39**	16.00 ± 0.45**	15.50 ± 0.31**
Day 88	2.88 ± 0.13[d]	11.20 ± 0.44**	17.22 ± 1.18**[c]	19.70 ± 0.62**	16.00 ± 0.42**
Heinz bodies (% erythrocytes)					
Day 25	0.0 ± 0.0	0.0 ± 0.0	1.5 ± 0.3**	14.4 ± 0.8**	21.2 ± 1.8**
Week 14	0.0 ± 0.0	0.2 ± 0.0**	4.8 ± 0.7**	6.8 ± 0.6**	16.0 ± 1.8**

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Data are presented as mean ± standard error. Statistical tests were performed on unrounded data. All 1,000 mg/kg rats died before the end of the study; no data are available for these groups.
[b] n=10
[c] n=9
[d] n=8

necrotic cells remained in the affected areas. Renal tubule dilatation was associated with variable dilatation of the renal cortical tubules. Atrophy of the splenic red pulp was characterized by decreased hematopoietic activity within the red pulp. Necrosis of the thymus was characterized by thymocytes with shrunken, pyknotic nuclei and karyorrhectic nuclear debris. In the nose, degeneration of the olfactory epithelium, and hyperplasia (males) or metaplasia (females) of the respiratory epithelium were observed in the 1,000 mg/kg groups as well as most of the other dosed groups.

Treatment-related histologic lesions occurred in the liver, nose, kidney, spleen, bone marrow, mesenteric lymph node, and forestomach of surviving groups of rats. These lesions are described below.

In the liver, there were significantly increased incidences of hepatocellular hypertrophy in males and females administered 125 mg/kg or greater (Table 4); the severity of this lesion was also generally increased. In males and females, there were significantly increased incidences of pigmentation in the 62.5, 125, 250, and 500 mg/kg groups. The incidences of hepatocyte necrosis in 62.5, 250, and 500 mg/kg females were significantly increased compared to the vehicle control incidences.

Hepatocellular hypertrophy consisted of large hepatocytes with abundant eosinophilic granular cytoplasm and some degree of nuclear enlargement (Plates 3 and 4). A coinciding increase in mitotic figures was not recorded separately. Hepatocellular necrosis (not designated as centrilobular) was characterized by liver lobules having randomly scattered hepatocytes with condensed eosinophilic cytoplasm and nuclear fragmentation. Pigmentation was diagnosed when there was an accumulation of golden-brown pigment, presumed to be hemosiderin, within Kupffer cells and was attributed to increased erythrocyte destruction. Hematopoietic cell proliferation was characterized by erythropoiesis – scattered small clusters of immature hematopoietic cells.

In the nose, dose-related increases in the incidences and severities of olfactory epithelium degeneration occurred in all dosed groups of males and females (Table 4). In the 250 and 500 mg/kg males and females, there were significantly increased incidences of olfactory epithelium metaplasia. The incidences of respiratory epithelium hyperplasia were significantly increased in males and females administered 125, 250, and 500 mg/kg. There were significantly increased incidences of respiratory epithelium squamous metaplasia in 62.5, 125, 250, and 500 mg/kg males and in females administered 125, 250, and 500 mg/kg. In males and females administered 125, 250, and 500 mg/kg, there were significantly increased incidences of hyperplasia of the glands underlying the olfactory epithelium (Bowman's glands).

Olfactory epithelial degeneration was characterized by disorganization, disruption or loss of epithelial cells, by various vacuoles within the epithelium, by degeneration and/or atrophy of the olfactory nerves, and by variable acute to chronic active inflammatory infiltrates (Plates 5 and 6). Olfactory epithelial metaplasia occurred when the normal olfactory epithelium was replaced by a ciliated respiratory epithelium. Hyperplasia of the respiratory epithelium was characterized by increased numbers of respiratory epithelial cells that formed a pseudostratified columnar epithelium. Metaplasia of the respiratory epithelium was recorded when a squamous epithelium replaced the normal respiratory epithelium. Hyperplasia of glands consisted of proliferations of the cells lining the glands, variable luminal/ductular dilatation, and occasional inflammatory infiltrates.

In the kidney, there were significantly increased incidences of pigmentation in males and females administered 62.5, 125, 250, and 500 mg/kg (Table 4). The incidences of papillary necrosis in 125, 250, and 500 mg/kg males and 250 mg/kg females were significantly greater than those in the vehicle controls. In males, there were significantly increased incidences of mineralization in the 125, 250, and 500 mg/kg groups. In females, there were significantly increased incidences of nephropathy in the 125, 250, and 500 mg/kg groups. Pigmentation was characterized by accumulation of orange-brown pigment consistent with hemosiderin. Papillary necrosis was characterized by patchy to diffuse acute necrosis of the renal papilla (Plates 7 and 8). Nephropathy was characterized by flattened basophilic renal tubular epithelial cells, luminal dilation, and a minimal thickening of the tubular basement membranes. Mineralization occurred within tubules of the deep cortex or medulla, often in association with papillary necrosis.

In the spleen, there were significantly increased incidences of congestion in all dosed groups of males and 125, 250, and 500 mg/kg females; lymphoid follicle atrophy in males administered 250 and 500 mg/kg and females administered 500 mg/kg; and capsular fibrosis in 125, 250, and 500 mg/kg males and females; the severities of these lesions also increased with dose (Table 4). There were also significantly increased incidences of mesothelial hypertrophy in 125 mg/kg males and 250 and 500 mg/kg males and females. The severities of hematopoietic cell proliferation and pigmentation in all dosed groups were increased compared to those of

TABLE 4
Incidences of Selected Nonneoplastic Lesions in Rats in the 3-Month Gavage Study of *N,N*-Dimethyl-*p*-toluidine[a]

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg
Male					
Liver[b]	10	10	10	10	10
Hepatocyte, Hypertrophy[c]	0	2 (1.0)[d]	9** (1.0)	10** (1.2)	10** (1.8)
Pigmentation	0	4* (1.0)	7** (1.0)	9** (1.0)	9** (1.0)
Nose	10	10	10	10	10
Glands, Hyperplasia	0	0	10** (1.8)	10** (2.1)	9** (2.1)
Olfactory Epithelium, Degeneration	0	5* (1.0)	10** (2.5)	10** (3.0)	10** (3.1)
Olfactory Epithelium, Metaplasia	0	0	0	9** (1.9)	9** (2.9)
Respiratory Epithelium, Hyperplasia	1 (1.0)	2 (1.0)	7** (1.4)	10** (1.5)	9** (1.8)
Respiratory Epithelium, Metaplasia, Squamous	0	8** (1.5)	10** (2.5)	10** (2.8)	9** (3.0)
Kidney	10	10	10	10	10
Mineralization	1 (1.0)	4 (1.0)	10** (1.3)	10** (1.8)	8** (2.1)
Pigmentation	0	10** (1.0)	10** (1.0)	10** (1.6)	9** (1.9)
Papilla, Necrosis	0	0	7** (1.3)	7** (1.7)	9** (2.4)
Spleen	10	10	10	10	10
Capsule, Fibrosis	1 (1.0)	5 (1.0)	10** (1.4)	10** (2.7)	9** (2.8)
Congestion	0	10** (1.2)	10** (1.8)	10** (2.4)	9** (3.0)
Hematopoietic Cell Proliferation	9 (1.0)	10 (2.0)	10 (2.0)	10 (1.9)	9 (1.8)
Lymphoid Follicle, Atrophy	0	0	0	8** (1.5)	10** (2.7)
Mesothelium, Hypertrophy	3 (1.3)	5 (1.2)	8* (1.5)	10** (1.5)	9** (1.8)
Pigmentation	10 (1.0)	10 (2.1)	10 (2.2)	10 (2.0)	9 (2.0)
Bone Marrow	10	10	10	10	10
Hyperplasia	0	10** (2.0)	10** (2.9)	10** (3.0)	10** (2.9)
Forestomach	10	10	10	10	10
Inflammation	0	0	1 (1.0)	0	5* (1.4)

TABLE 4
Incidences of Selected Nonneoplastic Lesions in Rats in the 3-Month Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg
Female					
Liver	10	10	10	10	10
Hepatocyte, Hypertrophy	0	1 (1.0)	7** (1.0)	9** (1.1)	10** (2.7)
Hepatocyte, Necrosis	1 (1.0)	6* (1.5)	5 (1.4)	7** (1.3)	6* (1.2)
Pigmentation	0	10** (1.0)	10** (1.0)	10** (1.8)	10** (1.9)
Nose	10	10	10	10	10
Glands, Hyperplasia	0	3 (1.0)	9** (1.7)	10** (1.9)	10** (2.0)
Olfactory Epithelium, Degeneration	0	7** (1.3)	10** (2.1)	10** (3.0)	10** (3.0)
Olfactory Epithelium, Metaplasia	0	0	0	7** (1.6)	10** (2.9)
Respiratory Epithelium, Hyperplasia	0	1 (1.0)	7** (1.1)	10** (1.7)	10** (1.7)
Respiratory Epithelium, Metaplasia, Squamous	0	0	6** (1.5)	10** (2.2)	10** (2.6)
Kidney	10	10	10	10	10
Nephropathy	2 (1.0)	2 (1.0)	9** (1.0)	10** (1.0)	10** (1.3)
Pigmentation	0	10** (1.0)	10** (1.0)	10** (1.0)	10** (1.6)
Papilla, Necrosis	0	0	0	6** (1.5)	2 (2.5)
Spleen	10	10	10	10	10
Capsule, Fibrosis	0	3 (1.0)	7** (1.3)	10** (2.2)	10** (2.7)
Congestion	0	2 (1.0)	10** (1.4)	10** (2.4)	10** (3.0)
Hematopoietic Cell Proliferation	10 (1.0)	10 (1.9)	10 (1.9)	10 (2.3)	10 (2.0)
Lymphoid Follicle, Atrophy	0	0	0	0	10** (1.3)
Mesothelium, Hypertrophy	0	1 (1.0)	2 (1.5)	9** (1.1)	9** (1.1)
Pigmentation	10 (1.0)	10 (2.0)	10 (2.0)	10 (1.9)	10 (2.0)
Bone Marrow	10	10	10	10	10
Hyperplasia	0	10** (1.9)	10** (2.7)	10** (3.0)	10** (3.0)
Lymph Node, Mesenteric	10	10	10	10	10
Atrophy	0	0	0	1 (2.0)	6** (2.2)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
** $P \leq 0.01$
[a] Data not shown for 1,000 mg/kg groups because all animals died during week 1.
[b] Number of animals with tissue examined microscopically
[c] Number of animals with lesion
[d] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

the vehicle control groups. The spleen of many dosed animals was noted to be enlarged macroscopically, and this finding correlated microscopically with congestion, which was characterized by increased numbers of red blood cells in the red pulp. Pigmentation in the spleen resulted from an accumulation of brown pigment consistent with hemosiderin deposition after erythrocyte destruction. Islands of proliferating hematopoietic cells within the red pulp characterized hematopoietic cell proliferation. Atrophy of lymphoid follicles was characterized by depletion of lymphocytes within lymphoid follicles. Capsular fibrosis was characterized by a variable thickening of the capsule due to an accumulation of fibrous connective tissue. Fibrotic areas often contained mononuclear cells; hypertrophy of the mesothelium often accompanied the finding.

In bone marrow, the incidences of hyperplasia in all dosed groups of male and female rats were significantly greater than those in the vehicle controls (Table 4).

Bone marrow hyperplasia was characterized by an expansion of marrow hematopoietic tissue with a concomitant decrease in marrow adipose tissue.

In the mesenteric lymph node, there were significantly increased incidences of atrophy in 500 mg/kg females (Table 4). Atrophy was characterized by a general depletion of lymphocytes and loss of follicles.

In the forestomach, the incidence of inflammation was significantly increased in 500 mg/kg males (Table 4); inflammation was characterized by mixed inflammatory infiltrates in the mucosa and submucosa.

Dose Selection Rationale: Based on mortality in the 1,000 mg/kg groups, decreased (more than 10%) final mean body weights in the 125, 250, and 500 mg/kg male groups, and treatment-related nonneoplastic lesions in the liver, nose, spleen, kidney, and bone marrow with increased severity at 125 mg/kg or greater, a high dose of 60 mg/kg *N*,*N*-dimethyl-*p*-toluidine was selected for the 2-year gavage study in rats. The low dose of 6 mg/kg was selected because this dose was reported to cause toxicity in humans (Potter *et al*., 1988). The doses selected for the 2-year gavage study in rats were 0, 6, 20, and 60 mg/kg, with a threefold dose spacing.

2-YEAR STUDY

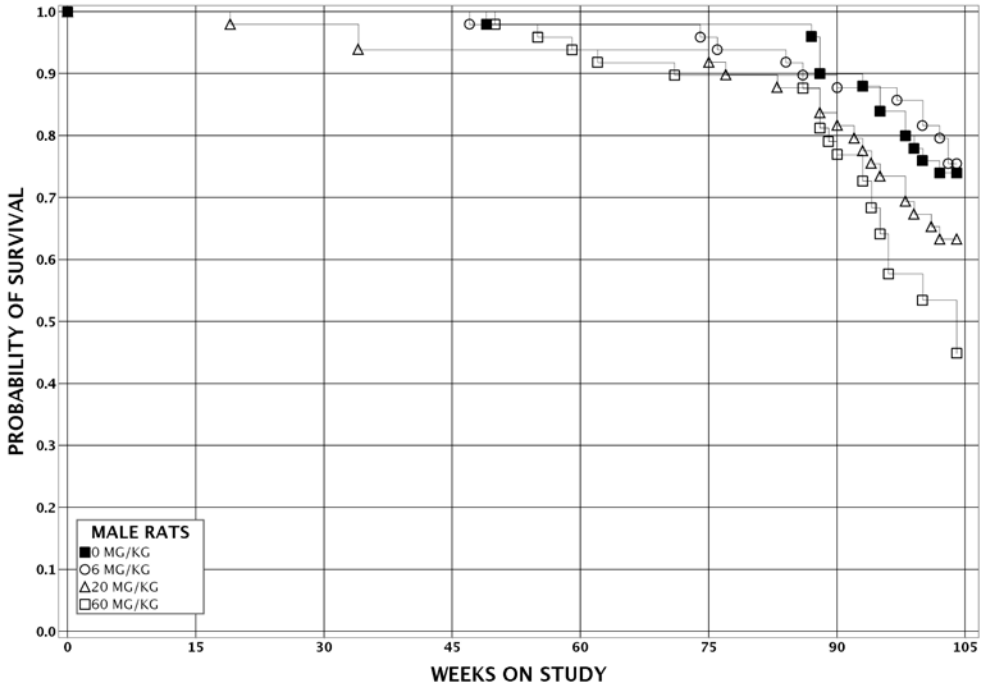
Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 5 and in the Kaplan-Meier survival curves (Figure 3). Survival of 60 mg/kg males was significantly less than that of the vehicle controls. Although the survival of 60 mg/kg females was decreased compared to the vehicle controls, the decrease was not statistically significant.

TABLE 5
Survival of Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Male				
Animals initially in study	50	50	50	50
Accidental deaths[a]	0	1	1	3
Moribund	11	7	11	7
Natural deaths	2	5	7	19
Animals surviving to study termination	37	37	31	21
Percent probability of survival at end of study[b]	74	76	63	45
Mean survival (days)[c]	702	687	657	652
Survival analysis[d]	P=0.001	P=1.000	P=0.290	P=0.006
Female				
Animals initially in study	50	50	50	50
Accidental deaths[a]	0	1	0	1
Moribund	14	3	8	8
Natural deaths	3	4	9	18
Animals surviving to study termination	33	42	33	23
Percent probability of survival at end of study	66	86	66	47
Mean survival (days)	704	701	707	651
Survival analysis	P=0.001	P=0.042N	P=1.000	P=0.062

[a] Censored from survival analyses
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal kill).
[d] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A lower mortality in a dosed group is indicated by **N**.



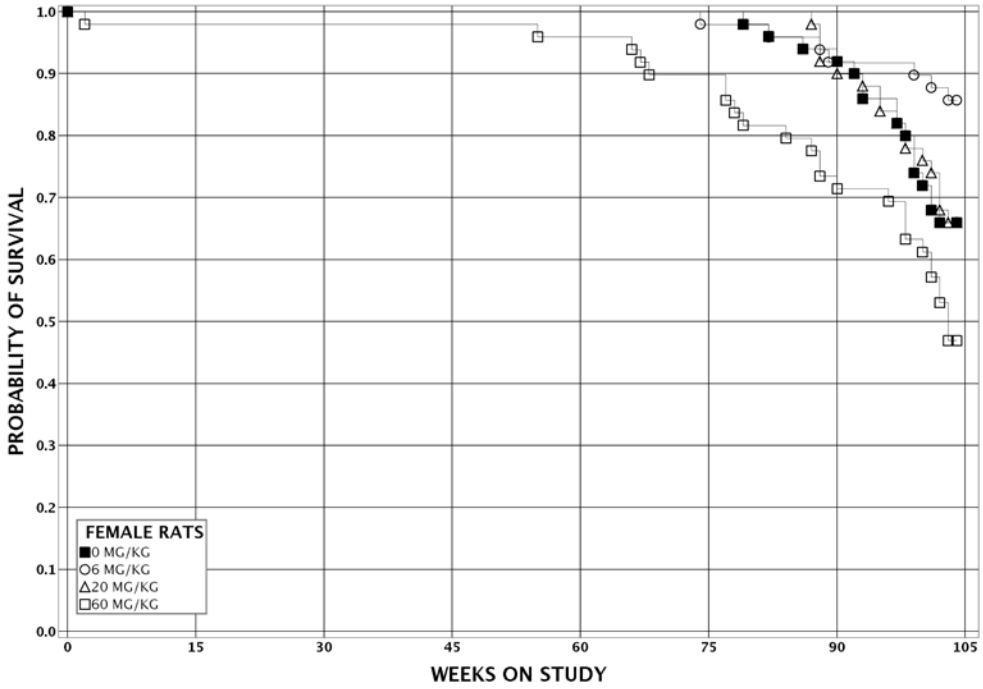


FIGURE 3
Kaplan-Meier Survival Curves for Rats Administered *N*,*N*-Dimethyl-*p*-toluidine by Gavage for 2 Years

Body Weights and Clinical Findings

The mean body weights of 60 mg/kg males were over 10% less than those of the vehicle controls after week 61 (day 421) and those of 60 mg/kg females were less than those of the vehicle controls after week 33 (day 225) (Figure 4; Tables 6 and 7). Clinical findings included signs of pallor in 60 mg/kg females and hyperactivity and boxing behavior in 20 mg/kg females and 60 mg/kg males and females. Hyperactivity and boxing behavior were first noticed during study month 8. All animals exhibited normal behavior prior to dosing. Boxing behavior, characterized by "kangaroo boxing" between cage mate pairs, was seen after dosing in 20 mg/kg females and 60 mg/kg males and females. In most months where this behavior was observed, the percentage of animals displaying this behavior was greater following the first dose of the week compared to the percentage following the last dose of the week. The percentage of 60 mg/kg females displaying boxing behavior decreased over the course of the study.

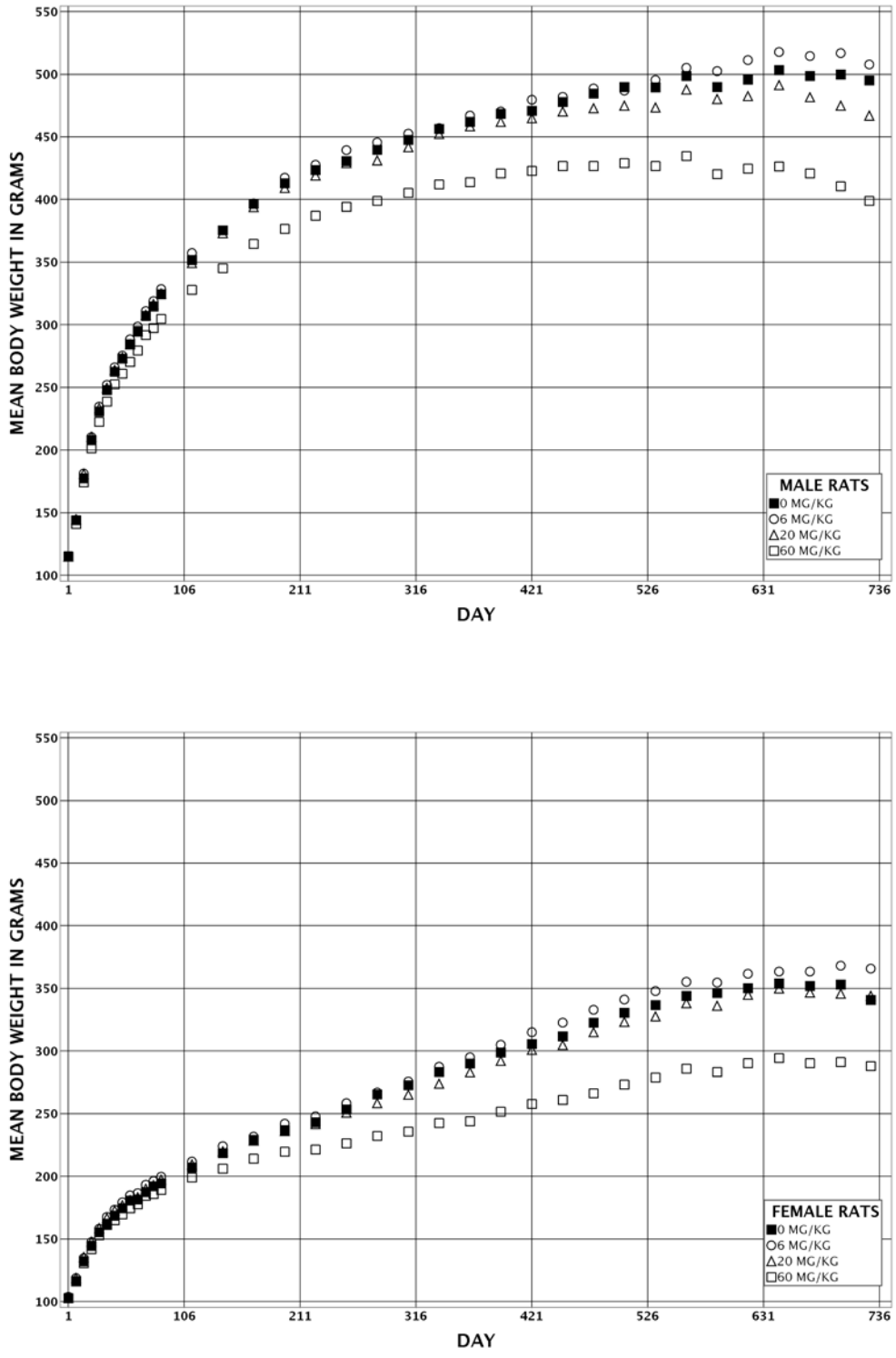


FIGURE 4
Growth Curves for Rats Administered *N,N*-Dimethyl-*p*-toluidine by Gavage for 2 Years

TABLE 6
Mean Body Weights and Survival of Male Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control		6 mg/kg			20 mg/kg			60 mg/kg		
Day	Av. Wt. (g)	No. of Survivors	Av. Wt (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt (g)	Wt. (% of Controls)	No. of Survivors
1	115	50	115	100	50	115	100	50	115	100	50
8	144	50	144	100	50	145	100	50	141	98	50
15	177	50	181	102	50	181	102	50	174	98	50
22	208	50	210	101	50	211	101	50	202	97	50
29	231	50	235	102	50	234	101	50	223	97	50
36	248	50	252	102	50	250	101	50	239	96	50
43	263	50	266	101	50	264	101	50	253	96	50
50	273	50	276	101	50	274	100	50	261	96	50
57	285	50	288	101	50	284	100	50	271	95	50
64	295	50	299	101	49	295	100	50	280	95	50
71	307	50	311	101	49	308	100	50	292	95	50
78	315	50	319	101	49	317	101	50	298	95	50
85	324	50	329	101	49	325	100	50	305	94	50
113	352	50	357	102	49	350	99	50	328	93	50
141	376	50	375	100	49	373	99	49	345	92	50
169	397	50	397	100	49	394	99	48	365	92	50
197	413	50	417	101	49	409	99	48	377	91	49
225	424	50	428	101	49	419	99	48	387	91	49
253	431	50	440	102	49	429	100	46	394	92	49
281	440	50	446	101	49	431	98	46	399	91	49
309	448	50	453	101	49	442	99	46	405	91	49
337	456	49	457	100	48	452	99	46	412	90	49
365	462	49	467	101	48	458	99	46	414	90	48
393	469	49	470	100	48	462	99	46	421	90	47
421	471	49	480	102	48	465	99	46	423	90	46
449	478	49	482	101	48	470	98	46	427	89	45
477	485	49	489	101	48	473	98	46	427	88	45
505	490	49	487	99	48	475	97	46	429	88	44
533	490	49	495	101	46	473	97	45	427	87	44
561	499	49	505	101	46	488	98	44	435	87	44
589	490	49	503	103	45	480	98	43	420	86	42
617	496	45	511	103	44	483	97	41	425	86	38
645	504	44	518	103	43	491	98	38	427	85	34
673	499	42	514	103	43	482	97	36	421	84	27
701	500	38	517	103	40	475	95	33	411	82	25
Mean for Weeks											
1-13	245		248	101		246	100		235	96	
14-52	415		419	101		411	99		379	91	
53-101	487		495	102		475	98		424	87	

TABLE 7
Mean Body Weights and Survival of Female Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control		6 mg/kg			20 mg/kg			60 mg/kg		
Day	Av. Wt. (g)	No. of Survivors	Av. Wt (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt (g)	Wt. (% of Controls)	No. of Survivors
1	103	50	104	101	50	103	100	50	103	101	50
8	117	50	119	102	50	119	102	50	116	100	50
15	132	50	135	102	50	135	102	50	131	99	49
22	145	50	148	102	50	148	102	50	142	98	49
29	156	50	158	102	50	159	102	50	153	98	49
36	162	50	168	103	50	167	103	50	162	100	49
43	169	50	173	103	49	173	102	50	165	98	49
50	175	50	179	103	49	177	101	50	170	97	49
57	181	50	185	102	49	181	100	50	175	97	49
64	182	50	187	103	49	183	101	50	178	98	49
71	188	50	193	103	49	190	101	50	185	98	49
78	192	50	196	102	49	193	101	50	186	97	49
85	195	50	200	103	49	197	101	50	189	97	49
113	207	50	212	102	49	210	101	50	199	96	49
141	219	50	224	103	49	220	101	50	206	94	49
169	229	50	232	101	49	229	100	50	214	94	49
197	237	50	242	102	49	236	100	50	220	93	49
225	243	50	248	102	49	242	99	50	222	91	49
253	254	50	259	102	49	251	99	50	226	89	49
281	266	50	267	101	49	258	97	50	232	88	49
309	273	50	276	101	49	265	97	50	236	87	49
337	283	50	288	102	49	274	97	50	243	86	49
365	290	50	295	102	49	283	98	50	244	84	49
393	299	50	305	102	49	292	98	50	252	84	47
421	306	50	315	103	49	301	99	50	258	84	47
449	312	50	323	104	49	305	98	50	261	84	47
477	323	50	333	103	49	315	98	50	266	83	44
505	331	50	341	103	49	323	98	50	273	83	44
533	337	50	348	103	48	328	97	50	279	83	43
561	344	49	355	103	48	338	98	50	286	83	40
589	347	48	355	102	47	336	97	50	283	82	39
617	350	47	362	103	45	345	99	46	290	83	36
645	354	44	364	103	45	350	99	45	295	83	35
673	352	41	364	103	45	347	99	41	290	83	34
701	353	34	368	104	43	346	98	38	291	82	29
Mean for Weeks											
1-13	161		165	102		163	101		158	98	
14-52	246		250	102		243	99		222	90	
53-101	331		341	103		324	98		275	83	

Hematology

The hematology findings in this 3-month interim evaluation were consistent with what occurred in the 3-month study. Increases in methemoglobin and Heinz bodies occurred in the 20 and 60 mg/kg male and female groups. Dose-related decreases occurred in the erythron characterized by decreases in hematocrit values, hemoglobin concentrations, and erythrocyte counts in the 20 and 60 mg/kg male and female groups (Tables 8 and F2). The erythron decreases were accompanied by trends toward erythrocyte macrocytosis and hypochromia evidenced by increases in the mean cell volume and decreases in the mean cell hemoglobin concentration values, respectively. Increases in reticulocyte counts demonstrated increased erythropoiesis in response to the decreased erythron. While the magnitudes of the erythron decreases were not sufficient to categorically classify these as anemias, the patterns of erythron changes were identical to what occurred in the 3-month study. At most, minimally decreased hemoglobin concentrations (decreased <5%), increased methemoglobin values (increased <20% in males only), and increased Heinz bodies (increased in females only) occurred in the 6 mg/kg groups.

TABLE 8
Selected Hematology Data for Rats at 3 Months in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine[a]

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
n	10	10	10	10
Male				
Hematocrit (%)	48.8 ± 0.5	48.4 ± 0.4	46.5 ± 0.3**	42.6 ± 0.3**
Hemoglobin (g/dL)	16.0 ± 0.2	15.6 ± 0.1*	14.7 ± 0.1**	13.2 ± 0.1**
Erythrocytes (10^6/µL)	9.10 ± 0.10	9.02 ± 0.06	8.53 ± 0.04**	7.61 ± 0.06**
Reticulocytes (10^6/µL)	0.25 ± 0.01	0.26 ± 0.01*	0.35 ± 0.01**	0.69 ± 0.02**
Mean cell volume (fL)	53.7 ± 0.2	53.6 ± 0.2	54.5 ± 0.2**	56.0 ± 0.1**
Mean cell hemoglobin (pg)	17.5 ± 0.1	17.3 ± 0.1	17.3 ± 0.1	17.3 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.7 ± 0.2	32.2 ± 0.2	31.6 ± 0.1**	30.9 ± 0.2**
Methemoglobin (g/dL)	0.77 ± 0.04	0.88 ± 0.03*	1.14 ± 0.03**	2.30 ± 0.03**
Methemoglobin (% hemoglobin)	4.70 ± 0.26	5.60 ± 0.22*	7.90 ± 0.18**	17.40 ± 0.22**
Heinz bodies (% erythrocytes)	0.0 ± 0.0	0.1 ± 0.1	0.7 ± 0.2**	3.7 ± 0.3**
Female				
Hematocrit (%)	46.9 ± 0.5	45.8 ± 0.6	44.2 ± 0.6**	41.3 ± 0.6**
Hemoglobin (g/dL)	15.8 ± 0.2	15.1 ± 0.2*	14.4 ± 0.2**	13.2 ± 0.1**
Erythrocytes (10^6/µL)	8.50 ± 0.09	8.31 ± 0.10	7.88 ± 0.08**	6.95 ± 0.09**
Reticulocytes (10^6/µL)	0.24 ± 0.01	0.24 ± 0.01	0.35 ± 0.01**	0.70 ± 0.02**
Mean cell volume (fL)	55.1 ± 0.2	55.1 ± 0.2	56.1 ± 0.3*	59.4 ± 0.2**
Mean cell hemoglobin (pg)	18.6 ± 0.1	18.2 ± 0.1*	18.3 ± 0.1	19.0 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.8 ± 0.2	33.1 ± 0.2*	32.6 ± 0.2**	32.0 ± 0.2**
Methemoglobin (g/dL)	0.80 ± 0.03	0.87 ± 0.03	1.21 ± 0.05**	2.26 ± 0.07**
Methemoglobin (% hemoglobin)	5.10 ± 0.23	5.60 ± 0.27	8.40 ± 0.31**	17.10 ± 0.41**
Heinz bodies (% erythrocytes)	0.0 ± 0.0	0.3 ± 0.2*	0.9 ± 0.3**	3.8 ± 0.2**

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
** Significantly different ($P \leq 0.01$) from the vehicle control group by Shirley's test
[a] Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of mononuclear cell leukemia and neoplasms and nonneoplastic lesions of the liver, nose, and thyroid gland; nonneoplastic lesions of the spleen, bone marrow, mesenteric lymph node, kidney, and forestomach; and neoplasms of the uterus, vagina, ovary, tongue, and mammary gland. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Liver: There were significant increases in the incidences of hepatocellular carcinoma and hepatocellular adenoma or carcinoma (combined) in 60 mg/kg males and females (Tables 9, A2, and B2). Hepatocellular carcinomas were characterized by enlarged hepatocytes arranged in a trabecular pattern with trabeculae at least three cells in width (Plates 9 and 10). Cells were variably sized and nuclei contained prominent nucleoli. Mitotic figures were occasionally observed. In contrast, hepatocellular adenomas tended to be smaller, more discrete masses that lacked a trabecular pattern. Hepatocellular adenomas caused compression of surrounding parenchyma and were composed of hepatocytes containing eosinophilic, basophilic or vacuolated cytoplasm. Hepatocellular adenomas and hepatocellular carcinomas are considered part of the same neoplastic process, and therefore were combined.

There were significantly increased incidences of eosinophilic focus in all dosed male groups and 20 and 60 mg/kg females, clear cell focus in all dosed female groups, and mixed cell focus in 60 mg/kg males and females (Tables 9, A4, and B4). Incidences of basophilic focus in all dosed groups of males and 20 and

TABLE 9
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Male				
Number Examined Microscopically	50	50	50	50
Basophilic Focus[a]	28	6**	0**	3**
Eosinophilic Focus	11	21*	21*	29**
Mixed Cell Focus	18	17	17	35**
Bile Duct, Fibrosis	21 (1.0)[b]	27 (1.0)	41** (1.1)	42** (1.5)
Bile Duct, Hyperplasia	40 (1.2)	42 (1.5)	44* (1.6)	44 (1.8)
Degeneration, Cystic	4 (1.3)	10 (1.4)	9 (1.3)	17** (1.3)
Hepatocyte, Hypertrophy	0	0	6* (1.5)	31** (1.5)
Hepatocellular Adenoma[c]	0	0	1	1
Hepatocellular Carcinoma[d]				
Overall rate[e]	0/50 (0%)	0/50 (0%)	1/50 (2%)	6/50 (12%)
Adjusted rate[f]	0.0%	0.0%	2.4%	14.9%
Terminal rate[g]	0/37 (0%)	0/37 (0%)	1/31 (3%)	2/21 (10%)
First incidence (days)	—[i]	—	727 (T)	612
Poly-3 test[h]	P<0.001	—[j]	P=0.479	P=0.009
Hepatocellular Adenoma or Carcinoma[k]				
Overall rate	0/50 (0%)	0/50 (0%)	2/50 (4%)	6/50 (12%)
Adjusted rate	0.0%	0.0%	4.8%	14.9%
Terminal rate	0/37 (0%)	0/37 (0%)	1/31 (3%)	2/21 (10%)
First incidence (days)	—	—	688	612
Poly-3 test	P<0.001	—	P=0.215	P=0.009

TABLE 9
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Female				
Number Examined Microscopically	50	50	50	49
Basophilic Focus	46	45	5**	6**
Clear Cell Focus	7	17*	24**	29**
Eosinophilic Focus	18	24	29*	32**
Mixed Cell Focus	14	20	17	26**
Bile Duct, Fibrosis	6 (1.2)	11 (1.0)	23** (1.0)	27** (1.1)
Bile Duct, Hyperplasia	10 (1.6)	21* (1.0)	27** (1.0)	43** (1.5)
Degeneration, Cystic	0	0	2 (1.0)	10** (1.2)
Hepatocyte, Hypertrophy	0	0	6* (1.3)	22** (1.3)
Hepatocyte, Necrosis	0	0	1 (2.0)	5* (1.8)
Hepatocellular Adenoma[l]				
Overall rate	0/50 (0%)	1/50 (2%)	1/50 (2%)	3/49 (6%)
Adjusted rate	0.0%	2.1%	2.2%	7.8%
Terminal rate	0/33 (0%)	1/42 (2%)	1/33 (3%)	2/23 (9%)
First incidence (days)	—	728 (T)	728 (T)	720
Poly-3 test	P=0.044	P=0.504	P=0.502	P=0.091
Hepatocellular Carcinoma, Multiple	0	0	0	1
Hepatocellular Carcinoma (includes multiple)[m]				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	4/49 (8%)
Adjusted rate	0.0%	0.0%	0.0%	10.4%
Terminal rate	0/33 (0%)	0/42 (0%)	0/33 (0%)	4/23 (17%)
First incidence (days)	—	—	—	728 (T)
Poly-3 test	P<0.001	—	—	P=0.041
Hepatocellular Adenoma or Carcinoma[n]				
Overall rate	0/50 (0%)	1/50 (2%)	1/50 (2%)	7/49 (14%)
Adjusted rate	0.0%	2.1%	2.2%	18.1%
Terminal rate	0/33 (0%)	1/42 (2%)	1/33 (3%)	6/23 (26%)
First incidence (days)	—	728 (T)	728 (T)	720
Poly-3 test	P<0.001	P=0.504	P=0.502	P=0.003

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
(T) Terminal kill
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year gavage studies with corn oil vehicle controls (mean ± standard deviation): 3/299 (1.0% ± 1.1%), range 0%-2%; all routes: 18/1,249 (1.4% ± 1.9%), range 0%-6%
[d] Historical incidence for corn oil gavage studies: 0/299; all routes: 5/1,249 (0.4% ± 1.0%), range 0%-4%
[e] Number of animals with neoplasm per number of animals with liver examined microscopically
[f] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[g] Observed incidence at terminal kill
[h] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
[i] Not applicable; no neoplasm in animal group
[j] Value of statistic cannot be computed.
[k] Historical incidence for corn oil gavage studies: 3/299 (1.0% ± 1.1%), range 0%-2%; all routes: 23/1,249 (1.8% ± 1.9%), range 0%-6%
[l] Historical incidence for corn oil gavage studies: 1/300 (0.3% ± 0.8%), range 0%-2%; all routes: 11/1,200 (0.9% ± 1.6%), range 0%-4%
[m] Historical incidence for corn oil gavage studies: 0/300; all routes: 1/1,200 (0.1% ± 0.4%), range 0%-2%
[n] Historical incidence for corn oil gavage studies: 1/300 (0.3% ± 0.8%), range 0%-2%; all routes: 12/1,200 (1.0% ± 1.6%), range 0%-4%

60 mg/kg females were significantly less than those of the vehicle controls. Incidences of cystic degeneration were significantly increased in 60 mg/kg males and females. In the bile ducts of the liver, fibrosis in 20 and 60 mg/kg males and females and hyperplasia in 20 mg/kg males and all dosed groups of females were significantly increased. In 20 and 60 mg/kg males and females, there were significantly increased incidences of hepatocellular hypertrophy. In 60 mg/kg females, there was a significantly increased incidence of hepatocellular necrosis.

Eosinophilic foci were typically composed of enlarged, eosinophilic hepatocytes that caused no or minimal compression of the surrounding parenchyma. In general, they tended to be smaller than hepatocellular adenomas. Mixed cell focus was diagnosed when a focus of altered hepatocytes was composed of two types of hepatocytes, and neither type made up more than 80% of the hepatocytes within the focus. Clear cell foci were characterized by hepatocytes with clear vacuoles, consistent with glycogen accumulation, within eosinophilic cytoplasm. Hepatocytes within a clear cell focus may have been enlarged, but were not necessarily so. Basophilic foci, a common background lesion in rats, were composed of hepatocytes that stained more basophilic than the surrounding or typical hepatocytes, and affected hepatocytes were often smaller in size. Cystic degeneration was characterized by multilocular cystic structures that often contained flocculent eosinophilic material. Foci of cystic degeneration were randomly located in the liver, and were occasionally observed within a focus of altered hepatocytes.

Bile duct hyperplasia was characterized by the presence of increased bile duct profiles in portal areas. Although bile duct hyperplasia is often associated with some fibrosis, bile duct fibrosis was recorded as a separate diagnosis during this study because the amount of fibrous connective tissue surrounding bile ducts was more than is typically associated with bile duct hyperplasia. In some occurrences, there was a focus of solid fibrous connective tissue that contained only one or two bile duct profiles.

Hypertrophied hepatocytes were up to three times larger than normal hepatocytes and the hypertrophy lacked a specific zonality, rather it was randomly distributed throughout the liver lobules. Hepatocellular necrosis ranged from scattered individual cell necrosis to large, coalescing areas of coagulative necrosis. Necrosis within a neoplasm was not recorded separately.

Nose: There were significantly increased incidences of transitional epithelium adenoma and transitional epithelium adenoma or carcinoma (combined) in 60 mg/kg males; transitional epithelium adenoma also occurred in the other dosed male groups and in 6 and 60 mg/kg females (Tables 10, A2, and B2). Transitional epithelium carcinoma occurred in two 60 mg/kg males. While the incidences of transitional epithelium adenoma in female rats were not statistically significant, these are very uncommon neoplasms. Transitional epithelial adenomas were typically small exophytic masses that arose from the transitional epithelium lining the nasoturbinates or the lateral wall between the maxilloturbinate and the nasoturbinate of the Level I nasal section (Plates 11 and 12). Neoplastic cells were moderately sized and polygonal with moderate amounts of lightly granular eosinophilic cytoplasm. Nuclei were also moderately sized and round to oval, with lightly stippled chromatin and one or two prominent basophilic to amphophilic nucleoli. Carcinomas were expansile masses that extended from the dorsolateral to ventral region of Level I, and resulted in obliteration of the maxilloturbinate (Plates 13 and 14). Like the cells in the transitional epithelial adenomas, cells in the carcinomas were polygonal with eosinophilic cytoplasm and prominent nucleoli, and appeared to arise from the transitional epithelium. The cells were arranged in sheets, or gland-like structures, and had a moderately vascular stroma. Mitotic figures were present but uncommon in the carcinomas (Plate 15), with none to one per five high power fields.

An adenoma of the glands underlying the olfactory epithelium (Bowman's glands) occurred in a 60 mg/kg male (Tables 10 and A1). While not statistically significant, this neoplasm is very unusual. It consisted of a mixed pattern of cells, with some cells containing basophilic cytoplasm with peripherally displaced nuclei and other cells containing single or a few large, colorless vacuoles (Plates 16 and 17). Still other cells contained brightly eosinophilic cytoplasm and appeared to be forming glands. Nests of cells were surrounded and separated by a delicate fibrovascular stroma.

In the transitional epithelium of the nose, the incidences of hyperplasia in 20 and 60 mg/kg males and females were significantly greater than those in the vehicle controls (Tables 10, A4, and B4). One 60 mg/kg female had degeneration of the transitional epithelium (Table B4). In the glands underlying the transitional epithelium, there were increased incidences of hyperplasia in 20 and 60 mg/kg males and females and dilatation in 20 mg/kg males and 60 mg/kg females. In addition, the severities of these lesions tended to increase with dose. Transitional epithelium hyperplasia was characterized by increased numbers of transitional epithelium cells lining the lateral wall, and less frequently the nasoturbinate, when compared to the same location in the vehicle controls. Degeneration of the

TABLE 10
Incidences of Neoplasms and Nonneoplastic Lesions of the Nose in Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Male				
Number Examined Microscopically	50	49	50	49
Glands, Olfactory Epithelium, Dilatation[a]	0	0	3 (1.0)[b]	49** (2.4)
Glands, Olfactory Epithelium, Hyperplasia	0	2 (1.0)	0	48** (1.9)
Glands, Olfactory Epithelium, Metaplasia	0	0	0	38** (1.5)
Glands, Olfactory Epithelium, Necrosis	0	0	0	22** (2.7)
Glands, Respiratory Epithelium, Dilatation	13 (1.0)	15 (1.0)	19 (1.0)	48** (1.6)
Glands, Respiratory Epithelium, Hyperplasia	0	8** (1.1)	8** (1.5)	41** (1.7)
Glands, Respiratory Epithelium, Metaplasia, Respiratory	29 (1.0)	39* (1.0)	39** (1.0)	47** (2.6)
Glands, Transitional Epithelium, Dilatation	0	0	5* (1.2)	3 (1.7)
Glands, Transitional Epithelium, Hyperplasia	0	1 (1.0)	24** (1.1)	40** (1.6)
Inflammation	35 (1.4)	40 (1.6)	38 (1.2)	48** (1.9)
Nerve, Atrophy	0	0	0	15** (1.3)
Olfactory Epithelium, Degeneration	0	0	1 (2.0)	47** (2.1)
Olfactory Epithelium, Hyperplasia, Basal Cell	0	1 (1.0)	2 (1.0)	38** (1.3)
Olfactory Epithelium, Metaplasia, Respiratory	4 (1.0)	9 (1.4)	9 (1.3)	40** (1.3)
Respiratory Epithelium, Hyperplasia	15 (1.2)	29** (1.5)	32** (1.3)	49** (1.6)
Transitional Epithelium, Hyperplasia	1 (2.0)	1 (1.0)	11** (1.1)	46** (1.7)
Glands, Olfactory Epithelium, Adenoma[c]	0	0	0	1
Transitional Epithelium, Adenoma[c]				
Overall rate[e]	0/50 (0%)	3/49 (6%)	2/50 (4%)	11/49 (22%)[d]
Adjusted rate[f]	0.0%	6.7%	4.8%	27.5%
Terminal rate[g]	0/37 (0%)	2/37 (5%)	1/31 (3%)	7/21 (33%)
First incidence (days)	—[i]	713	688	582
Poly-3 test[h]	P<0.001	P=0.113	P=0.215	P<0.001
Transitional Epithelium, Carcinoma[j]				
Overall rate	0/50 (0%)	0/49 (0%)	0/50 (0%)	2/49 (4%)
Adjusted rate	0.0%	0.0%	0.0%	5.1%
Terminal rate	0/37 (0%)	0/37 (0%)	0/31 (0%)	0/21 (0%)
First incidence (days)	—	—	—	669
Poly-3 test	P=0.033	—[k]	—	P=0.203
Transitional Epithelium, Adenoma or Carcinoma[j]				
Overall rate	0/50 (0%)	3/49 (6%)	2/50 (4%)	13/49 (27%)
Adjusted rate	0.0%	6.7%	4.8%	32.3%
Terminal rate	0/37 (0%)	2/37 (5%)	1/31 (3%)	7/21 (33%)
First incidence (days)	—	713	688	582
Poly-3 test	P<0.001	P=0.113	P=0.215	P<0.001

TABLE 10
Incidences of Neoplasms and Nonneoplastic Lesions of the Nose in Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Female				
Number Examined Microscopically	50	49	50	49
Glands, Olfactory Epithelium, Dilatation	0	0	0	48** (2.4)
Glands, Olfactory Epithelium, Hyperplasia	0	0	4 (1.0)	47** (1.9)
Glands, Olfactory Epithelium, Metaplasia	0	0	0	42** (1.3)
Glands, Olfactory Epithelium, Necrosis	0	0	0	18** (2.8)
Glands, Respiratory Epithelium, Dilatation	5 (1.0)	12 (1.0)	27** (1.1)	47** (1.2)
Glands, Respiratory Epithelium, Hyperplasia	6 (1.2)	9 (1.0)	22** (1.3)	45** (1.6)
Glands, Respiratory Epithelium, Metaplasia, Respiratory	17 (1.1)	33** (1.1)	44** (1.8)	47** (2.0)
Glands, Transitional Epithelium, Dilatation	0	0	0	9** (1.4)
Glands, Transitional Epithelium, Hyperplasia	0	4 (1.0)	12** (1.2)	24** (1.4)
Inflammation	23 (1.3)	24 (1.4)	22 (1.1)	45** (1.5)
Nerve, Atrophy	0	0	0	4* (1.8)
Olfactory Epithelium, Degeneration	0	0	1 (1.0)	46** (2.0)
Olfactory Epithelium, Hyperplasia, Basal Cell	0	0	0	25** (1.2)
Olfactory Epithelium, Metaplasia, Respiratory	4 (1.5)	6 (1.5)	1 (2.0)	21** (1.2)
Respiratory Epithelium, Hyperplasia	10 (1.0)	13 (1.4)	11 (1.1)	41** (1.3)
Transitional Epithelium, Hyperplasia	0	1 (1.0)	6* (1.0)	33** (1.1)
Transitional Epithelium, Adenoma[l]				
Overall rate	0/50 (0%)	1/49 (2%)	0/50 (0%)	2/49 (4%)
Adjusted rate	0.0%	2.2%	0.0%	5.1%
Terminal rate	0/33 (0%)	1/42 (2%)	0/33 (0%)	0/23 (0%)
First incidence (days)	—	728 (T)	—	625
Poly-3 test	P=0.127	P=0.503	—	P=0.203

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
(T) Terminal kill
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence of nose adenoma (epithelium unspecified) for 2-year gavage studies with corn oil vehicle controls (mean ± standard deviation): 0/299; all routes: 0/1,248
[d] A single incidence of adenoma in the glands underlying the olfactory epithelium occurred in an animal that also had an adenoma in the transitional epithelium.
[e] Number of animals with neoplasm per number of animals with nose examined microscopically
[f] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[g] Observed incidence at terminal kill
[h] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
[i] Not applicable; no neoplasm in animal group
[j] No historical control data available
[k] Value of statistic cannot be computed.
[l] Historical incidence of nose adenoma (epithelium unspecified) for corn oil gavage studies: 0/299; all routes: 1/1,196 (0.1% ± 0.4%), range 0%-2%

transitional epithelium was characterized by decreased cellularity and disorganization of the transitional epithelium. Hyperplasia of the glands underlying the transitional epithelium was characterized by increased numbers of submucosal glands in the lateral wall region when compared to the number of glands in the same region in the vehicle controls.

In the olfactory epithelium of the nose, there were significantly increased incidences of degeneration, basal cell hyperplasia, and respiratory metaplasia in 60 mg/kg males and females (Tables 10, A4, and B4). In the glands underlying the olfactory epithelium, the incidences of hyperplasia, dilatation, metaplasia, and necrosis in 60 mg/kg males and females were significantly greater than those in the vehicle controls. Degeneration of the olfactory epithelium was characterized by disorganization of the olfactory epithelium, with increased intercellular spaces and vacuolization or apoptosis of individual cells (Plates 18 and 19) and was observed most commonly in Level II and/or III of affected rats. Basal cell hyperplasia of the olfactory epithelium consisted of minimal to mild increased numbers of basal epithelial cells along the basal lamina; this lesion was observed most frequently in Level III, and less commonly in Level II, of affected rats (Plates 20 and 21). Replacement of olfactory epithelium by respiratory epithelium was recorded as respiratory metaplasia of the olfactory epithelium and occurred in Levels II and III of affected rats. Two 60 mg/kg females had squamous metaplasia of the olfactory epithelium (Table B4); this lesion consisted of replacement of the olfactory epithelium by attenuated epithelium. Hyperplasia of the glands underlying the olfactory epithelium was characterized by the presence of increased numbers of glands, while dilatation consisted of minimal to moderately dilated glands that often contained eosinophilic material, inflammatory cells and sloughed epithelial cells (Plates 22, 23, and 24). These glands were usually lined by nonciliated cuboidal to attenuated epithelial cells. Metaplasia of the glands was characterized by glands in the olfactory region that were lined by ciliated cuboidal to columnar epithelium (Plate 24). Necrosis of the glands consisted of glands that were lined by variably sized, eosinophilic to vacuolated, epithelial cells with pyknotic nuclei (Plate 25). Affected glands often contained cell debris or sloughed epithelial cells.

In the respiratory epithelium of the nose, the incidences of hyperplasia were significantly increased in all dosed male groups and 60 mg/kg females (Tables 10, A4, and B4). In the glands underlying the respiratory epithelium, there were significantly increased incidences of hyperplasia in all dosed male groups and 20 and 60 mg/kg females, dilatation in 20 mg/kg females and 60 mg/kg males and females, and respiratory metaplasia in all dosed groups of males and females. Hyperplasia of the respiratory epithelium was characterized by increased numbers of respiratory epithelial cells, which often formed invaginations into the underlying submucosa, making it difficult to distinguish hyperplasia of the respiratory epithelium from hyperplasia of the glands underlying the respiratory epithelium. In some instances, the glands underlying the respiratory epithelium were dilated, and in others, were characterized by metaplasia. Respiratory metaplasia of the glands was diagnosed when the glands, most commonly at the olfactory/respiratory junction of Level II, were lined by ciliated columnar epithelium.

There were significantly increased incidences of inflammation of the nose and atrophy of the nerves underlying the olfactory epithelium in 60 mg/kg males and females (Tables 10, A4, and B4). Inflammation of the nose consisted predominantly of neutrophils with fewer macrophages, and occasional plasma cells and eosinophils in the lamina propria of the olfactory, respiratory, and/or transitional epithelial regions, and within the nasal cavity. Atrophy of the nerves underlying the olfactory epithelium was often associated with degeneration of the olfactory epithelium and was characterized by the loss of nerve bundles in the olfactory regions, primarily in Level III, and less often in Level II.

There were negative trends in the incidences of hyaline droplet accumulation in the respiratory and olfactory epithelium in dosed groups of males and females (Tables A4 and B4). Hyaline droplet accumulation consisted of brightly eosinophilic droplets or globules within the cytoplasm of the epithelial cells.

Thyroid Gland: There were increased incidences of follicular cell adenoma or carcinoma (combined) in all dosed groups of males (Tables 11, A1, and A2). Follicular cell carcinomas occurred only in treated males, not control males. While the increased incidences were not statistically significant, the incidence in 60 mg/kg males exceeded the historical control ranges for corn oil gavage studies and for all routes of exposure (Tables 11 and A3c). In 20 mg/kg females, there was an increased incidence of follicular cell adenoma (Tables 11 and B1). Although not statistically significant, the incidence of follicular cell adenoma in 20 mg/kg females exceeded the historical control ranges for corn oil gavage studies and for all routes. However, unlike in males, no follicular cell neoplasms occurred in 60 mg/kg females, but follicular cell adenoma occurred in one control female. The highest incidence of follicular cell neoplasms in females occurred in the 20 mg/kg group, but was only one more than that of the control group. Follicular cell adenomas were usually a single, well-circumscribed expansile mass that caused compression of the adjacent

TABLE 11
Incidences of Neoplasms of the Thyroid Gland in Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Male				
Follicular Cell Adenoma[a]				
Overall rate[b]	1/50 (2%)	0/49 (0%)	1/50 (2%)	3/49 (6%)
Adjusted rate[c]	2.2%	0.0%	2.4%	7.7%
Terminal rate[d]	1/37 (3%)	0/37 (0%)	0/31 (0%)	3/21 (14%)
First incidence (days)	727 (T)	—[f]	680	727 (T)
Poly-3 test[e]	P=0.060	P=0.506N	P=0.738	P=0.248
Follicular Cell Carcinoma[g]				
Overall rate	0/50 (0%)	2/49 (4%)	1/50 (2%)	2/49 (4%)
Adjusted rate	0.0%	4.5%	2.4%	5.1%
Terminal rate	0/37 (0%)	2/37 (5%)	1/31 (3%)	2/21 (10%)
First incidence (days)	—	727 (T)	727 (T)	727 (T)
Poly-3 test	P=0.261	P=0.230	P=0.479	P=0.202
Follicular Cell Adenoma or Carcinoma[h]				
Overall rate	1/50 (2%)	2/49 (4%)	2/50 (4%)	4/49 (8%)
Adjusted rate	2.2%	4.5%	4.8%	10.3%
Terminal rate	1/37 (3%)	2/37 (5%)	1/31 (3%)	4/21 (19%)
First incidence (days)	727 (T)	727 (T)	680	727 (T)
Poly-3 test	P=0.088	P=0.489	P=0.465	P=0.132
Female				
Follicular Cell Adenoma[i]				
Overall rate	1/49 (2%)	1/47 (2%)	2/47 (4%)	0/45 (0%)
Adjusted rate	2.2%	2.2%	4.6%	0.0%
Terminal rate	0/33 (0%)	1/42 (2%)	2/33 (6%)	0/23 (0%)
First incidence (days)	701	728 (T)	728 (T)	—
Poly-3 test	P=0.397N	P=0.760	P=0.491	P=0.539N

(T) Terminal kill

[a] Historical incidence for 2-year gavage studies with corn oil vehicle controls (mean ± standard deviation): 6/299 (2.0% ± 1.3%), range 0%-4%; all routes: 13/1,239 (1.0% ± 1.7%), range 0%-6%

[b] Number of animals with neoplasm per number of animals with thyroid gland examined microscopically

[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[d] Observed incidence at terminal kill

[e] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in a dose group is indicated by **N**.

[f] Not applicable; no neoplasm in animal group

[g] Historical incidence for corn oil gavage studies: 3/299 (1.0% ± 1.7%), range 0%-4%; all routes: 10/1,239 (0.8% ± 1.5%), range 0%-4%

[h] Historical incidence for corn oil gavage studies: 9/299 (3.0% ± 2.1%), range 0%-6%; all routes: 23/1,239 (1.9% ± 2.2%), range 0%-6%

[i] Historical incidence for corn oil gavage studies: 3/298 (1.0% ± 1.1%), range 0%-2%; all routes: 8/1,186 (0.7% ± 1.0%), range 0%-2%

parenchyma and contained papillary infoldings of epithelial cells. Carcinomas tended to be larger and less uniform, with more pleomorphism of the cells and the growth patterns. The incidences of follicular cell hyperplasia were not increased in males or females (Tables A4 and B4).

Hematopoietic System (Spleen, Bone Marrow and Mesenteric Lymph Node): In the spleen, there were significantly increased incidences of congestion in 60 mg/kg males and all dosed female groups, hematopoietic cell proliferation in all dosed male and female groups, and pigmentation in all dosed male groups and 60 mg/kg females (Tables 12, A4, and B4). Compared to that in the vehicle control group, the severities of pigmentation increased in dosed groups of males. The incidences of lymphoid follicle atrophy in 6 mg/kg males and 60 mg/kg males and females, capsule fibrosis in 60 mg/kg males and females, and mesothelial hypertrophy of the capsule in 60 mg/kg males and all dosed female groups were significantly greater than those in the vehicle controls. In 60 mg/kg males, there was also a significantly (P≤0.01) increased incidence of red pulp atrophy (vehicle control, 0/50; 6 mg/kg, 0/50; 20 mg/kg, 1/50; 60 mg/kg, 8/50; Table A4). Congestion was characterized by erythrocytes distending the sinusoids of the red pulp, while hematopoietic cell proliferation consisted of minimal to moderate increases in the number of erythroid and myeloid precursors, along with megakaryotes, scattered throughout the red pulp. Pigmentation consisted of dark brown granules, consistent with hemosiderin, within macrophages. Atrophy of the lymphoid follicle was diagnosed when there was a minimal to moderate decrease in the size of lymphoid follicles when compared to the vehicle controls, and atrophy of the red pulp was diagnosed when there was a mild to moderate reduction of red pulp with compressed vascular spaces. Capsular fibrosis was characterized by thickening of the capsule by fibrous connective tissue and small numbers of mononuclear cells when compared to the vehicle controls (Plates 26 and 27). Hypertrophy of the mesothelium was characterized by enlarged mesothelial cells lining the splenic capsule.

In bone marrow, there were significantly increased incidences of hyperplasia in 20 and 60 mg/kg males and 60 mg/kg females (Tables 12, A4, and B4). Bone marrow hyperplasia was characterized by an expansion of marrow hematopoietic tissue due to increased numbers of hematopoietic cells, with a concomitant decrease in marrow adipose tissue.

In the mesenteric lymph node, the incidences of histiocytic cellular infiltrates in 20 and 60 mg/kg males were significantly greater than that in the vehicle controls (Tables 12 and A4). This lesion consisted of an increased number of histiocytes (macrophages) within the lymph node, usually within the medullary sinuses.

Kidney: There were significantly increased incidences of nephropathy in all dosed female groups and increases in the severity of the lesion in all dosed male groups (Tables 12 and A4). The incidences of pigmentation in all dosed male groups and 60 mg/kg females were significantly greater than those in the vehicle control groups. In addition, the incidences of hyperplasia of the transitional epithelium of the renal pelvis were significantly increased ($P<0.05$) in 20 mg/kg males (1/50, 2/50, 6/50, 5/50; Table A4) and females (2/50, 3/50, 8/50, 6/50; Table B4). Nephropathy was characterized by the presence of regenerative cortical tubules with occasionally thickened basement membranes, variable amounts of interstitial connective tissue, and mononuclear cellular infiltrates. Accumulation of dark brown granular pigment was observed within cortical epithelial cells. Hyperplasia of the transitional epithelium consisted of proliferations of transitional epithelial cells, often in papillary or frond-like proliferations, along the renal pelvis, and was considered secondary to nephropathy.

Forestomach: There were significantly increased incidences of ulcer and hyperplasia in 20 and 60 mg/kg males and inflammation in 60 mg/kg males (Tables 12 and A4). Ulceration of the forestomach was diagnosed when there was full-thickness necrosis of the squamous epithelium, and included lesions in which a focus of necrotic cells remained in the affected area. Ulcers were often associated with inflammation and hyperplasia. Inflammation was characterized by mixed inflammatory infiltrates in the mucosa and submucosa, and epithelial hyperplasia was characterized by focal areas of increased layers of squamous epithelium.

Other Organs: In female rats, the incidences of uterine stromal polyp (3/50, 9/50, 4/50, 8/50) and uterine stromal polyp or stromal sarcoma (combined) (3/50, 9/50, 5/50, 8/50) were increased in the 6 and 60 mg/kg groups (Tables B1 and B2). The incidence of uterine stromal polyp in the 6 mg/kg group exceeded the historical control range for corn oil gavage studies [34/300 (11.3% ± 4.7%), range 6% to 16%] but not for all routes of exposure [189/1,200 (15.8% ± 6.6%), range 4% to 34%]. There was one vaginal polyp in a 6 mg/kg female (Table B1). Granulosa cell tumors occurred in the ovary of three 6 mg/kg females (Tables B1 and B2) and the combined incidence of these benign or malignant neoplasms in this group exceeded the historical control

TABLE 12
Incidences of Selected Nonneoplastic Lesions in Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Male				
Bone Marrow[a]	50	50	50	50
Hyperplasia[b]	17 (2.5)[c]	13 (2.5)	28** (2.1)	50** (2.7)
Forestomach	50	50	50	50
Hyperplasia	0	3 (1.7)	5* (2.2)	11** (2.2)
Inflammation	1 (2.0)	5 (1.6)	5 (2.6)	7* (2.6)
Ulcer	0	2 (2.0)	5* (2.6)	6** (2.0)
Kidney	50	50	50	50
Nephropathy	49 (1.4)	49 (2.0)	48 (2.5)	49 (2.7)
Pigmentation	24 (1.2)	46** (1.0)	37** (1.2)	44** (1.6)
Mesenteric Lymph Node	50	50	50	50
Infiltration Cellular, Histiocyte	21 (1.1)	23 (1.4)	30* (1.3)	34** (1.5)
Spleen	50	50	50	50
Capsule, Fibrosis	1 (2.0)	0	2 (1.5)	46** (1.8)
Capsule, Hypertrophy, Mesothelium	0	1 (1.0)	3 (1.0)	39** (1.1)
Congestion	1 (2.0)	0	0	39** (1.9)
Hematopoietic Cell Proliferation	34 (1.0)	44* (1.1)	42* (1.5)	44** (1.3)
Lymphoid Follicle, Atrophy	0	5* (2.2)	2 (1.5)	19** (2.0)
Pigmentation	36 (1.1)	48** (1.7)	47** (2.1)	48** (2.0)
Female				
Bone Marrow	50	50	50	50
Hyperplasia	18 (2.8)	13 (2.5)	18 (2.7)	49** (2.6)
Kidney	50	50	50	50
Nephropathy	28 (1.1)	38* (1.2)	38* (1.2)	41** (1.8)
Pigmentation	41 (1.0)	45 (1.0)	43 (1.0)	49** (1.4)
Spleen	50	50	50	50
Capsule, Fibrosis	8 (1.1)	0	8 (1.1)	41** (1.3)
Capsule, Hypertrophy, Mesothelium	1 (1.0)	14** (1.0)	10** (1.0)	16** (1.1)
Congestion	0	9** (1.1)	26** (1.3)	28** (1.8)
Hematopoietic Cell Proliferation	32 (1.6)	45** (1.8)	47** (1.9)	42** (1.7)
Lymphoid Follicle, Atrophy	1 (2.0)	2 (3.0)	0	28** (2.4)
Pigmentation	44 (2.0)	47 (2.1)	47 (2.5)	49* (2.2)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

ranges for corn oil gavage studies [1/300 (0.3% ± 0.8%), range 0% to 2%] and for all routes of exposure [8/1,198 (0.7% ± 1.1%), range 0% to 4%]. There was no dose response for any of the above neoplasms, and no pairwise statistical significance, therefore, they were not considered to be treatment related.

In the tongue, the incidences of squamous cell papilloma or squamous cell carcinoma (combined) were increased in 6 and 60 mg/kg males and 60 mg/kg females (males: vehicle control, 0/50; 6 mg/kg, 1/50; 20 mg/kg, 0/50; 60 mg/kg, 1/50; females: 1/50, 0/50, 0/50, 2/50; Tables A1 and B1). While the incidences of these neoplasms were not significantly increased, the incidence in 60 mg/kg females exceeded the historical control ranges for corn oil gavage studies [2/300 (0.7% ± 1.0%), range 0% to 2%] and all routes of exposure [5/1,200 (0.4% ± 0.8%), range 0% to 2%] and the incidences in 6 and 60 mg/kg males exceeded the historical control range for corn oil gavage studies (0/299). However, the lack of a dose response or statistical significance led to the conclusion that these neoplasms were not associated with exposure to *N,N*-dimethyl-*p*-toluidine. The tongue is not examined microscopically unless a gross lesion is observed; therefore, the historical control values may not be as robust for tongue neoplasms as for other tissues.

There were negative trends in the incidences of mononuclear cell leukemia in males and females and the incidences in all dosed groups were significantly less than those in the vehicle control groups (males: 14/50, 1/50, 2/50, 0/50; females: 15/50, 2/50, 1/50, 1/50; Tables A2 and B2).

In the mammary gland of 60 mg/kg females, there was a significantly decreased incidence of fibroadenoma (29/50, 26/50, 26/50, 11/50; Tables A1 and A3) that was considered to be due to decreased body weights in this dose group (Haseman *et al*., 1997; Stout *et al*., 2008).

MICE
3-MONTH STUDY

All 250 mg/kg male and female mice died before day 10 except for one male mouse that survived until terminal kill (Table 13). Three males and two females administered 125 mg/kg died before the end of the study. The final mean body weight of 125 mg/kg males and the mean body weight gains of 125 mg/kg males and females were significantly less than those of the vehicle controls (Table 13 and Figure 5). Clinical findings associated with administration of *N,N*-dimethyl-*p*-toluidine included abnormal breathing, thinness, lethargy, cyanosis, and ruffled fur in 125 and 250 mg/kg males and females.

TABLE 13
Survival and Body Weights of Mice in the 3-Month Gavage Study of *N,N*-Dimethyl-*p*-toluidine[a]

Dose (mg/kg)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	10/10	23.1 ± 0.3	33.6 ± 1.4	10.5 ± 1.2	
15	10/10	22.6 ± 0.2	35.2 ± 0.5	12.6 ± 0.4	105
30	10/10	22.8 ± 0.3	33.0 ± 1.0	10.2 ± 0.9	98
60	10/10	22.8 ± 0.4	33.1 ± 1.0	10.3 ± 0.8	99
125	7/10[c]	22.6 ± 0.2	29.5 ± 0.4*	7.0 ± 0.2*	88
250	1/10[d]	22.7 ± 0.3	26.5	5.2	79
Female					
0	10/10	18.4 ± 0.2	27.7 ± 0.7	9.4 ± 0.6	
15	10/10	18.9 ± 0.2	29.4 ± 0.5	10.5 ± 0.4	106
30	10/10	18.2 ± 0.3	28.2 ± 0.9	9.9 ± 0.7	102
60	10/10	18.5 ± 0.2	27.8 ± 0.6	9.4 ± 0.5	100
125	8/10[e]	19.0 ± 0.3	26.2 ± 0.3	6.9 ± 0.3**	95
250	0/10[e]	18.3 ± 0.3	—	—	—

* Significantly different (P≤0.05) from the vehicle control group by Williams' test
** P≤0.01
[a] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[b] Number of animals surviving at 14 weeks/number initially in group
[c] Weeks of deaths: 2, 2, 11
[d] Weeks of deaths: 1, 1, 1, 1, 1, 1, 1, 2
[e] Week of deaths: 1

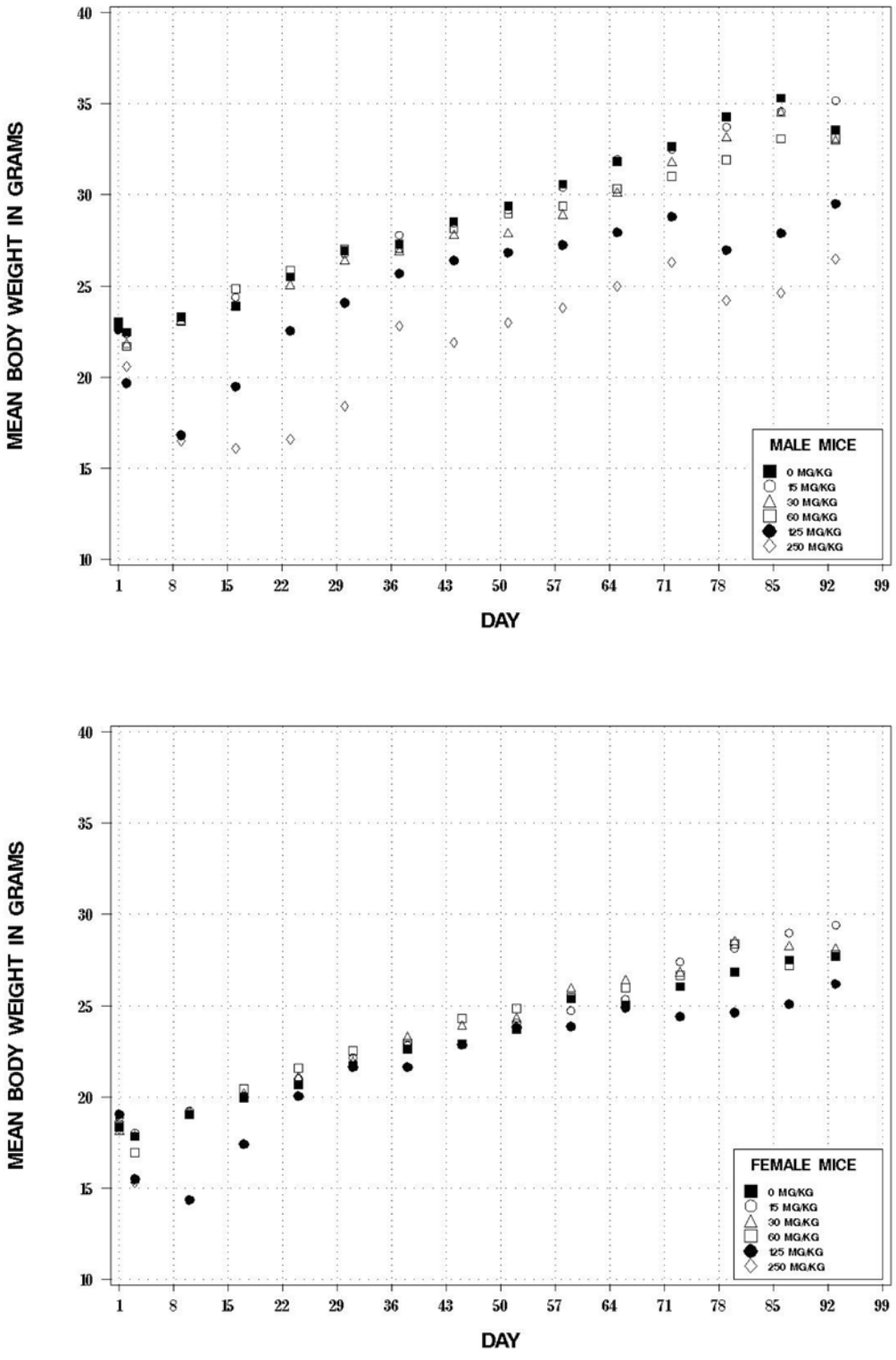


FIGURE 5
Growth Curves for Mice Administered *N,N*-Dimethyl-*p*-toluidine by Gavage for 3 Months

The hematology data for mice are presented in Tables 14 and F3. At equivalent doses, mice demonstrated similar, but less severe erythron changes compared to rats. Methemoglobin values were minimally increased in 30 mg/kg or greater males and females. Heinz bodies demonstrated small increases in 60 mg/kg females, 125 mg/kg males and females, and the lone surviving 250 mg/kg male. In fact, for female mice no erythron changes were detected up to the highest remaining dose (125 mg/kg) and for males, inconsistent and minor decreases in hematocrit values, hemoglobin concentrations, and erythrocyte counts and increased reticulocyte counts occurred in the 60 mg/kg and greater groups (including the lone surviving 250 mg/kg male).

The absolute and relative liver weights of all dosed male and female groups and the absolute and relative lung weights of 125 mg/kg males and females were significantly greater than those of the vehicle controls (Table G2). The absolute and relative thymus weights of 125 mg/kg females were significantly less than those of the vehicle controls.

There were no significant differences in any of the reproductive organ weights or sperm parameters of male mice at any dose (Table H3). There were no changes in the proportion of regularly cycling females, estrous cycle length, or percentage of time spent in the individual stages of the estrous cycle of female mice at any

TABLE 14
Selected Hematology Data for Mice in the 3-Month Gavage Study of *N,N*-Dimethyl-*p*-toluidine[a]

	Vehicle Control	15 mg/kg	30 mg/kg	60 mg/kg	125 mg/kg
Male					
n	10	10	10	10	7
Hematocrit (%)	46.6 ± 0.6	43.7 ± 0.5*	45.4 ± 0.6	43.5 ± 0.5**	44.7 ± 0.5
Hemoglobin (g/dL)	16.4 ± 0.3	15.5 ± 0.2	16.0 ± 0.3	15.0 ± 0.1**	15.3 ± 0.1**
Erythrocytes (10^6/μL)	10.82 ± 0.18	10.18 ± 0.14*	10.63 ± 0.15	10.14 ± 0.12*	10.27 ± 0.10
Reticulocytes (10^6/μL)	0.25 ± 0.01	0.24 ± 0.01	0.26 ± 0.01	0.27 ± 0.01	0.28 ± 0.01*
Mean cell volume (fL)	43.1 ± 0.2	42.9 ± 0.2	42.8 ± 0.1	42.9 ± 0.2	43.5 ± 0.4
Mean cell hemoglobin (pg)	15.2 ± 0.1	15.2 ± 0.1	15.0 ± 0.2	14.8 ± 0.1*	15.0 ± 0.1
Mean cell hemoglobin concentration (g/dL)	35.3 ± 0.3	35.4 ± 0.3	35.1 ± 0.4	34.5 ± 0.2	34.4 ± 0.3
Methemoglobin (g/dL)	0.35 ± 0.02	0.36 ± 0.02	0.42 ± 0.02*	0.47 ± 0.02**	0.61 ± 0.03**
Methemoglobin (% hemoglobin)	2.10 ± 0.10	2.50 ± 0.17	2.80 ± 0.13**	3.10 ± 0.10**	4.00 ± 0.22**
Heinz bodies (% erythrocytes)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.1**
Female					
n	10	9	10	10	8
Hematocrit (%)	44.9 ± 0.4	43.8 ± 0.6	45.5 ± 0.6	44.9 ± 0.4	46.4 ± 0.7
Hemoglobin (g/dL)	15.8 ± 0.3	15.5 ± 0.2	16.1 ± 0.2	15.7 ± 0.1	16.1 ± 0.2
Erythrocytes (10^6/μL)	10.42 ± 0.11	10.13 ± 0.15	10.57 ± 0.14	10.41 ± 0.07	10.64 ± 0.12
Reticulocytes (10^6/μL)	0.26 ± 0.02	0.26 ± 0.02	0.24 ± 0.02	0.24 ± 0.02	0.31 ± 0.02
Mean cell volume (fL)	43.1 ± 0.1	43.2 ± 0.1	43.0 ± 0.1	43.1 ± 0.1	43.6 ± 0.2
Mean cell hemoglobin (pg)	15.1 ± 0.2	15.3 ± 0.1	15.2 ± 0.1	15.1 ± 0.0	15.2 ± 0.0
Mean cell hemoglobin concentration (g/dL)	35.1 ± 0.4	35.4 ± 0.2	35.3 ± 0.2	35.1 ± 0.1	34.8 ± 0.2*
Methemoglobin (g/dL)	0.32 ± 0.01	0.34 ± 0.02	0.43 ± 0.02**	0.53 ± 0.02**	0.58 ± 0.03**
Methemoglobin (% hemoglobin)	2.10 ± 0.10	2.22 ± 0.15	2.60 ± 0.16*	3.40 ± 0.16**	3.88 ± 0.13**
Heinz bodies (% erythrocytes)	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.2 ± 0.1**	0.5 ± 0.1**

* Significantly different (P≤0.05) from the vehicle control group by Dunn's or Shirley's test
** P≤0.01
[a] Data are presented as mean ± standard error. Statistical tests were performed on unrounded data. No data are presented for the 250 mg/kg groups due to high mortality.

dose (Table H4). Therefore, *N*,*N*-dimethyl-*p*-toluidine was not considered to have the potential to be a reproductive toxicant in male or female mice.

Many of the histological findings in 250 mg/kg male and female mice were not observed in lower dose groups and were considered to be associated with stress and/or nonspecific toxicity. These lesions included hepatocellular fatty change and necrosis of the liver; chronic active inflammation of the nose; and necrosis and/or atrophy of the lymph nodes, spleen, and thymus. In mild cases, fatty change of the liver consisted of small, colorless, discrete vacuoles within the cytoplasm of hepatocytes. Although this change was diffuse, it was more pronounced in the centrilobular zone. Centrilobular hepatocytes enlarged by coalescing colorless vacuoles characterized more severe occurrences of fatty change. Cells in these animals often looked degenerative, including having nuclear changes such as peripheralized chromatin and shrunken nuclei. Necrosis of the liver typically occurred in random, irregular patches of either brightly eosinophilic individualized hepatocytes lacking nuclei or containing pyknotic nuclei or areas devoid of cellular detail. Hemorrhage was present in some of the necrotic areas. Hepatocellular necrosis also occurred as individual cell necrosis associated with fatty change. Chronic active inflammation of the nose consisted of a mixture of inflammatory cells, predominantly within the nasal cavity itself, but to a lesser extent, in the epithelium and lamina propria. Occasionally, sloughed cells or cell debris were present in the lumen. Atrophy was characterized by a general depletion of lymphocytes and a loss of follicles in the lymph nodes, a decreased cortical cellularity due to decreased numbers of thymocytes in the thymus, and an overall decrease in organ size due to white and red pulp depletion in the spleen. Necrosis of the spleen and lymph nodes was characterized by necrotic lymphocytes in follicles, which were evidenced by scattered remnants of pyknotic and karyorrhectic nuclei.

In the lung, incidences of degeneration of the bronchiolar epithelium and peribronchiolar chronic active inflammation were observed in the 250 mg/kg groups and were increased in the 125 mg/kg groups. Necrosis of the tracheal epithelium was observed in most of the males and all of the females exposed to 250 mg/kg and two males and females exposed to 125 mg/kg.

Treatment-related histological lesions occurred in the liver, lung, trachea, nose, and thymus of mice administered 125 mg/kg or less. These lesions are described below.

In the liver, the severities of cytoplasmic vacuolization of the hepatocytes were increased in dosed groups of male and female mice (Table 15). Cytoplasmic vacuolization was characterized by irregular, colorless to lightly eosinophilic areas in the cytoplasm consistent with glycogen accumulation that frequently resulted in hepatocyte enlargement.

In the lung, there were significantly increased incidences of bronchiolar epithelium degeneration and peribronchiolar chronic active inflammation and bronchiolar epithelium regeneration in 125 mg/kg males and females, and histocytic infiltrates of the alveoli in 125 mg/kg females (Table 15). Degeneration of the bronchiole epithelium was characterized in some mice by loss of apical cytoplasm or apical cytoplasmic blebbing, variations in cell size, and architectural disorganization. In other mice, there was an absence of epithelial cells lining the bronchioles or the presence of necrotic epithelial cells with shrunken pyknotic or karyorrhectic nuclei and eosinophilic cytoplasm. Sloughed cells could occasionally be seen in bronchiolar lumens. Attempts at regeneration ranged from epithelial cells with attenuated cytoplasm extending to cover denuded areas to multiple layers of proliferating epithelial cells lining the bronchioles (Plates 28 and 29). Peribronchiolar chronic active inflammation was composed of a mixture of inflammatory cells, including neutrophils, lymphocytes and plasma cells, with fewer macrophages, although alveolar histiocytes were prominent in a few animals. Typically, the inflammatory reaction was centered on the bronchioles.

In tracheas in which necrosis occurred, there were denuded areas due to the loss of epithelial cells or the presence of shrunken epithelial cells with pyknotic nuclei.

In the nose, there were significantly increased incidences of glandular hyperplasia in 125 mg/kg males and females (Table 15). In the olfactory epithelium of the nose, there were significantly increased incidences of degeneration in 125 mg/kg males and 60 and 125 mg/kg females and metaplasia in 125 mg/kg males and females. Glandular hyperplasia was characterized by increased profiles of glands in affected areas; these glands were often dilated and sometimes contained cell debris. Degeneration of the olfactory epithelium was characterized by a loss of nuclei and a thinning of the layer, a disorganization of the layer, or cytoplasmic vacuoles within the olfactory epithelial cells. Metaplasia of the olfactory epithelium was of the respiratory

TABLE 15
Incidences of Selected Nonneoplastic Lesions in Mice in the 3-Month Gavage Study of *N,N*-Dimethyl-*p*-toluidine[a]

	Vehicle Control	15 mg/kg	30 mg/kg	60 mg/kg	125 mg/kg
Male					
Liver[b]	10	10	10	10	10
Hepatocyte, Vacuolization Cytoplasmic[c]	9 (2.0)[d]	10 (3.0)	9 (2.6)	10 (2.6)	7 (2.6)
Lung	10	10	10	10	10
Bronchiole, Epithelium, Degeneration	0	0	0	1 (2.0)	10** (2.8)
Bronchiole, Epithelium, Regeneration	0	0	0	1 (2.0)	9** (2.7)
Peribronchiolar, Inflammation, Chronic Active	0	0	0	0	9** (2.2)
Nose	10	10	10	10	10
Glands, Hyperplasia	0	0	0	0	7** (2.0)
Olfactory Epithelium, Degeneration	0	0	0	0	9** (2.9)
Olfactory Epithelium, Metaplasia	0	0	0	0	6** (2.3)
Thymus	10	10	10	10	10
Thymocyte, Necrosis	0	0	0	0	8** (2.0)
Female					
Liver	10	10	10	10	10
Hepatocyte, Vacuolization Cytoplasmic	10 (1.0)	10 (2.2)	9 (2.1)	9 (2.3)	8 (2.6)
Lung	10	10	10	10	10
Alveolus, Infiltration Cellular, Histiocyte	0	0	0	0	7** (2.0)
Bronchiole, Epithelium, Degeneration	0	0	0	0	6** (2.5)
Bronchiole, Epithelium, Regeneration	0	0	1 (2.0)	1 (1.0)	7** (3.1)
Peribronchiolar, Inflammation, Chronic Active	0	1 (2.0)	1 (2.0)	1 (2.0)	10** (2.3)
Nose	10	10	10	10	10
Glands, Hyperplasia	0	0	0	0	7** (2.1)
Olfactory Epithelium, Degeneration	0	0	0	5* (1.8)	8** (2.5)
Olfactory Epithelium, Metaplasia	0	0	0	0	4* (2.5)
Thymus	10	10	10	10	10
Thymocyte, Necrosis	0	0	1 (1.0)	0	10** (2.0)

* Significantly different (P≤0.05) from the vehicle control group by the Fisher exact test
** P≤0.01
[a] Data not shown for 250 mg/kg groups because of mortality during week 1 and week 2.
[b] Number of animals with tissue examined microscopically
[c] Number of animals with lesion
[d] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

type, and consisted of ciliated cuboidal to columnar epithelium replacing the normal olfactory epithelium. Nerve bundles in these areas were atrophic.

In the thymus, the incidences of thymocyte necrosis in 125 mg/kg males and females were significantly increased compared to those in the vehicle controls (Table 15). Necrosis was evidenced by scattered remnants of pyknotic and karyorrhectic nuclei found primarily in the cortical region.

Dose Selection Rationale: Based on treatment-related mortality in the 250 mg/kg group and toxicity in the liver, lung, trachea, nose, and thymus in the 125 mg/kg group, the highest *N*,*N*-dimethyl-*p*-toluidine dose selected for the 2-year gavage study in mice was 60 mg/kg. As in rats, 6 mg/kg was selected as the low dose because this dose was reported to cause toxicity in humans (Potter *et al*., 1988). The doses selected for the 2-year gavage study in mice were 0, 6, 20, and 60 mg/kg with a threefold dose spacing.

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 16 and in the Kaplan-Meier survival curves (Figure 6). Survival of the 60 mg/kg female group was significantly less than that of the vehicle control group; survival of dosed groups of males was similar to that of the vehicle control group.

TABLE 16
Survival of Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Male				
Animals initially in study	50	50	50	50
Accidental deaths[a]	2	0	0	0
Moribund	5	4	11	14
Natural deaths	9	10	8	0
Animals surviving to study termination	34[e]	36	31	36
Percent probability of survival at end of study[b]	71	72	62	72
Mean survival (days)[c]	665	702	683	694
Survival analysis[d]	P=1.000	P=1.000	P=0.448	P=1.000
Female				
Animals initially in study	50	50	50	50
Accidental deaths[a]	0	1	1	2
Moribund	3	1	7	10
Natural deaths	4	8	3	6
Animals surviving to study termination	43	40	39	32
Percent probability of survival at end of study	86	82	80	67
Mean survival (days)	715	696	690	656
Survival analysis	P=0.014	P=0.763	P=0.553	P=0.034

[a] Censored from survival analyses
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal kill).
[d] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns.
[e] Includes one animal that died during the last week of the study

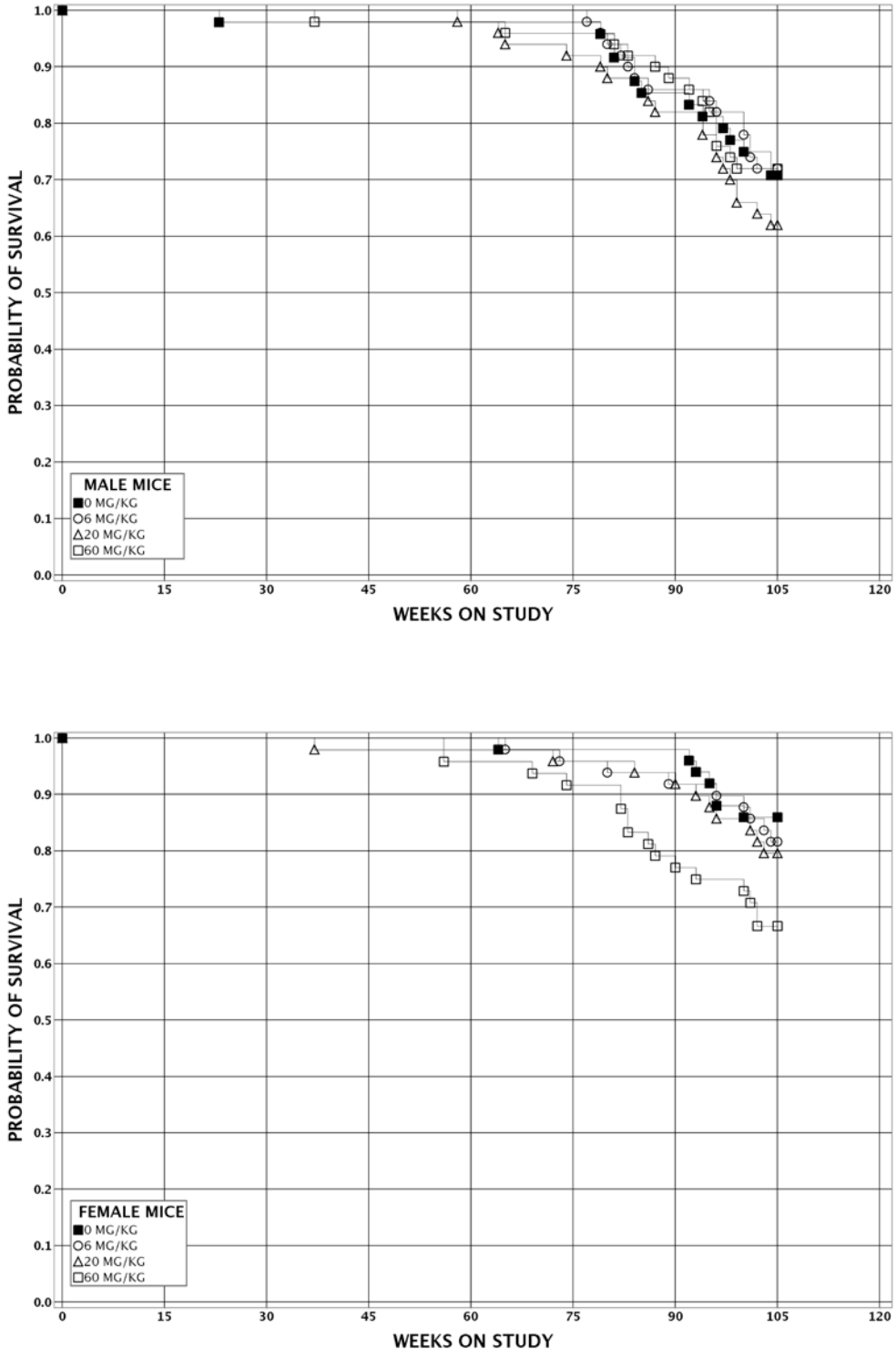


FIGURE 6
Kaplan-Meier Survival Curves for Mice Administered *N,N*-Dimethyl-*p*-toluidine by Gavage for 2 Years

Body Weights and Clinical Findings

The mean body weights of 60 mg/kg males were over 10% less than those of the vehicle controls after week 89 (day 617), and those of 60 mg/kg females were less than those of the vehicle controls after week 65 (day 449) (Tables 17 and 18; Figure 7). No clinical findings related to chemical administration were observed.

TABLE 17
Mean Body Weights and Survival of Male Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control		6 mg/kg			20 mg/kg			60 mg/kg		
Day	Av. Wt. (g)	No. of Survivors	Av. Wt (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt (g)	Wt. (% of Controls)	No. of Survivors
1	23.0	50	23.0	100	50	23.0	100	50	23.0	100	50
8	23.2	48	23.1	100	50	23.2	100	50	22.0	95	50
15	24.3	48	24.4	100	50	24.6	101	50	24.4	100	50
22	26.1	48	26.2	100	50	26.4	101	50	26.1	100	50
29	26.6	48	26.7	100	50	26.9	101	50	27.1	102	50
36	27.4	48	27.5	101	50	27.6	101	50	27.9	102	50
43	28.6	48	28.3	99	50	28.5	100	50	28.6	100	50
50	30.7	48	30.3	99	50	30.8	100	50	30.3	99	50
57	31.7	48	31.3	99	50	31.5	100	50	31.4	99	50
64	32.4	48	32.0	99	50	32.5	100	50	32.4	100	50
71	33.7	48	33.4	99	50	34.2	102	50	33.6	100	50
78	34.7	48	34.7	100	50	35.6	103	50	34.9	101	50
85	35.9	48	35.8	100	50	36.7	102	50	35.8	100	50
113	40.8	48	40.2	99	50	40.6	100	50	39.1	96	50
141	44.0	48	44.0	100	50	44.2	101	50	41.3	94	50
169	45.7	47	45.6	100	50	46.0	101	50	42.6	93	50
197	48.2	47	48.0	100	50	49.2	102	50	44.0	91	50
225	49.5	47	49.1	99	50	50.6	102	50	45.7	93	50
253	50.9	47	50.6	99	50	52.0	102	50	47.9	94	50
281	51.9	47	51.6	99	50	53.0	102	50	49.7	96	49
309	51.7	47	51.2	99	50	53.4	103	50	51.0	99	49
337	52.4	47	52.7	101	50	53.2	102	50	51.2	98	49
365	53.2	47	53.6	101	50	54.9	103	50	52.0	98	49
393	54.5	47	54.4	100	50	56.1	103	50	53.9	99	49
421	54.9	47	54.8	100	50	56.6	103	49	54.0	99	49
449	54.3	47	54.2	100	50	56.3	104	47	54.2	100	48
477	55.1	47	54.8	100	50	57.2	104	47	55.4	101	48
505	55.2	47	55.2	100	50	57.2	104	47	55.7	101	48
533	55.1	47	54.7	99	50	57.5	104	46	54.0	98	48
561	55.2	45	55.4	101	47	57.0	103	44	52.9	96	48
589	55.1	42	55.7	101	44	53.5	97	44	52.0	94	46
617	55.7	41	55.3	99	43	54.6	98	41	51.1	92	45
645	55.6	40	54.7	98	43	52.6	95	41	49.3	89	43
673	55.5	39	54.4	98	41	51.3	92	37	47.9	86	38
701	55.3	36	53.3	97	39	50.4	91	33	46.5	84	36
Mean for Weeks											
1-13	29.1		29.0	100		29.3	101		29.0	100	
14-52	48.3		48.1	100		49.1	102		45.8	95	
53-101	55.0		54.7	99		55.0	100		52.2	95	

TABLE 18
Mean Body Weights and Survival of Female Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control		6 mg/kg			20 mg/kg			60 mg/kg		
Day	Av. Wt. (g)	No. of Survivors	Av. Wt (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt (g)	Wt. (% of Controls)	No. of Survivors
1	18.7	50	18.6	100	50	18.6	100	50	18.5	99	50
8	18.4	50	18.6	101	49	18.6	101	50	16.7	91	50
15	19.2	50	19.4	101	49	19.5	102	49	19.4	101	50
22	20.7	50	20.4	98	49	21.0	101	49	20.6	100	50
29	21.0	50	21.1	100	49	21.6	103	49	21.8	104	50
36	22.3	50	22.4	101	49	22.1	99	49	22.4	100	50
43	22.9	50	23.0	100	49	23.0	100	49	23.5	102	49
50	24.1	50	24.3	101	49	23.8	99	49	24.4	101	49
57	24.7	50	25.3	102	49	25.0	101	49	25.7	104	48
64	26.2	50	26.6	101	49	26.2	100	49	26.0	99	48
71	27.1	50	27.2	100	49	27.0	100	49	27.5	102	48
78	28.4	50	28.8	102	49	28.2	99	49	27.9	98	48
85	29.1	50	29.4	101	49	29.7	102	49	29.3	101	48
113	32.8	50	33.6	102	49	33.8	103	49	32.4	99	48
141	36.7	50	37.4	102	49	37.1	101	49	36.0	98	48
169	39.3	50	40.3	103	49	40.3	103	49	39.2	100	48
197	43.3	50	43.7	101	49	43.1	100	49	41.5	96	48
225	46.2	50	46.9	102	49	46.6	101	49	44.3	96	48
253	48.3	50	49.5	102	49	48.5	100	48	47.5	98	48
281	51.9	50	52.8	102	49	51.2	99	48	50.4	97	48
309	54.6	50	54.9	100	49	54.5	100	48	50.8	93	48
337	57.4	50	57.8	101	49	57.5	100	48	52.8	92	48
365	59.0	50	58.1	99	49	59.5	101	48	52.2	89	48
393	59.9	50	61.7	103	49	61.2	102	48	55.3	92	46
421	62.9	50	64.0	102	49	63.4	101	48	56.3	90	46
449	63.9	49	64.4	101	48	65.6	103	48	57.3	90	46
477	65.1	49	65.1	100	48	67.3	103	48	56.7	87	46
505	66.6	49	65.3	98	48	68.5	103	47	56.6	85	45
533	66.2	49	65.8	99	47	69.2	105	47	55.5	84	44
561	65.5	49	66.4	101	46	68.6	105	47	55.2	84	44
589	64.3	49	66.3	103	46	68.6	107	46	52.0	81	40
617	63.7	49	66.0	104	46	67.5	106	46	51.3	81	38
645	62.3	47	65.1	104	45	67.4	108	45	49.8	80	36
673	62.6	44	59.6	95	44	66.1	106	42	46.4	74	36
701	62.8	43	61.0	97	43	63.9	102	42	45.0	72	34
Mean for Weeks											
1-13	23.3		23.5	101		23.4	100		23.4	100	
14-52	45.6		46.3	102		45.8	100		43.9	96	
53-101	63.4		63.8	101		65.9	104		53.0	84	

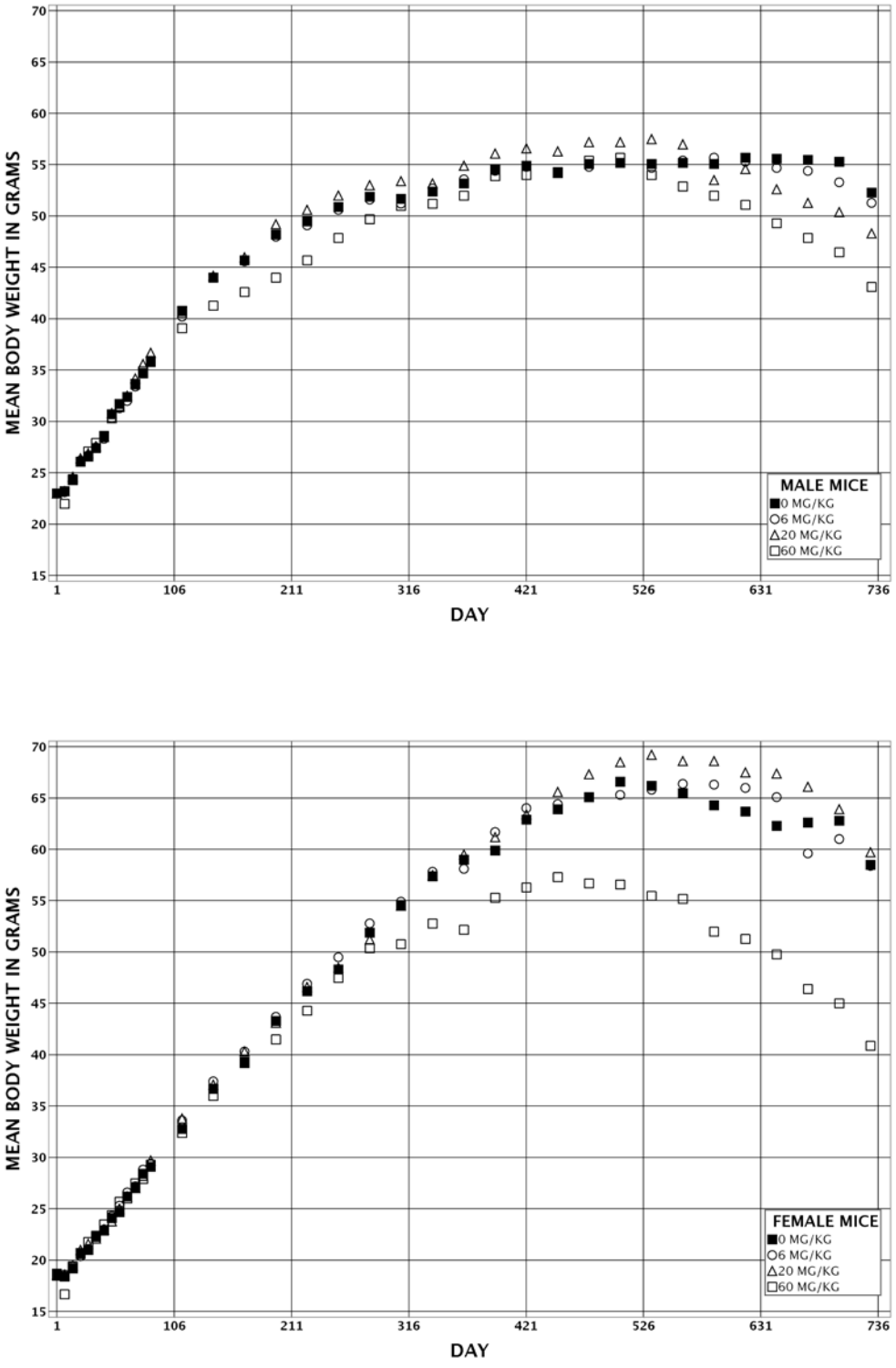


FIGURE 7
Growth Curves for Mice Administered *N,N*-Dimethyl-*p*-toluidine by Gavage for 2 Years

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and nonneoplastic lesions of the liver, lung, and forestomach; nonneoplastic lesions of the nose, olfactory lobe, spleen, bone marrow, mandibular and mesenteric lymph nodes; and neoplasms of the Harderian gland. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix C for male mice and Appendix D for female mice.

Liver: There were significantly increased incidences of hepatocellular adenoma in 20 and 60 mg/kg females and hepatocellular carcinoma in 60 mg/kg males and all dosed female groups (Tables 19, C2, and D2). The incidences of multiple hepatocellular adenoma were increased in 20 and 60 mg/kg males and females, and the incidences of multiple hepatocellular carcinoma were increased in 20 and 60 mg/kg males and 60 mg/kg females. There were also significantly increased incidences of hepatoblastoma in males receiving 20 and 60 mg/kg and females receiving 60 mg/kg. The incidences of hepatocellular adenoma or carcinoma (combined) and hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) were significantly increased in males and females receiving 20 and 60 mg/kg. Hepatocellular adenomas typically consisted of a well-circumscribed mass, which caused compression of the surrounding hepatic parenchyma, and was composed of a monotonous population of hepatocytes. Most commonly, hepatocellular carcinomas were large lesions that contained trabeculae at least three cells thick. Areas of necrosis and hemorrhage were common. Hepatoblastomas, unlike the previously described proliferative lesions, were composed of cells with scant amounts of basophilic cytoplasm, and oval, vesicular to open-faced nuclei. These cells were often arranged in nests and whorls.

The incidences of eosinophilic focus in 20 and 60 mg/kg males and females were significantly greater than those in the vehicle controls (Tables 19, C4, and D4). Dosed mice had multiple eosinophilic foci more frequently than the vehicle controls. There were significantly decreased incidences of mixed cell focus and clear cell focus in 60 mg/kg males. In contrast to hepatocellular adenomas, eosinophilic foci did not cause compression circumferentially and tended to be smaller, although this was not always the case. Mixed cell foci were diagnosed when a focus of altered hepatocytes was composed of two types of hepatocytes, and neither type made up more than 80% of the hepatocytes within the focus. Clear cell foci were characterized by hepatocytes with clear vacuoles, consistent with glycogen accumulation, within eosinophilic cytoplasm. Hepatocytes within a clear cell focus may have been enlarged, but were not necessarily so.

In all dosed groups of males and females, there were significantly increased incidences of hepatocellular hypertrophy (Tables 19, C4, and D4). There were also significantly increased incidences of diffuse fatty change in 60 mg/kg females and necrosis in 6 and 60 mg/kg females. In males, the severity of necrosis was increased in dosed groups although the incidences were not. In hepatocellular hypertrophy, the affected hepatocytes were large, with homogenous eosinophilic cytoplasm. Diffuse fatty change was characterized by microvesicles within hepatocytes. In some animals, the fatty change appeared to be centrilobular in distribution, but the change typically lacked a specific zonality. Necrosis typically consisted of focal areas of coagulative necrosis surrounded by mixed inflammatory cells; but occasionally, necrosis was more widespread and

TABLE 19
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Male				
Number Examined Microscopically	50	50	50	50
Clear Cell Focus[a]	15	22	15	7*
Eosinophilic Focus	25	30	39**	43**
Mixed Cell Focus	21	25	17	12*
Hepatocyte, Hypertrophy	1 (1.0)[b]	9** (1.2)	11** (1.9)	16** (2.1)
Necrosis	9 (1.6)	8 (2.5)	7 (1.9)	10 (2.0)
Hepatocellular Adenoma, Multiple	17	19	27*	26*
Hepatocellular Adenoma (includes multiple)[c]	29	34	37	36
Hepatocellular Carcinoma, Multiple	7	7	16*	22**
Hepatocellular Carcinoma (includes multiple)[d]				
Overall rate[e]	22/50 (44%)	25/50 (50%)	30/50 (60%)	36/50 (72%)
Adjusted rate[f]	48.9%	52.2%	65.1%	75.7%
Terminal rate[g]	16/34 (47%)	17/36 (47%)	19/31 (61%)	27/36 (75%)
First incidence (days)	548	539	442	562
Poly-3 test[h]	P=0.002	P=0.458	P=0.084	P=0.005
Hepatocellular Adenoma or Carcinoma[i]				
Overall rate	38/50 (76%)	44/50 (88%)	47/50 (94%)	48/50 (96%)
Adjusted rate	83.1%	90.6%	98.0%	98.6%
Terminal rate	28/34 (82%)	34/36 (94%)	31/31 (100%)	36/36 (100%)
First incidence (days)	548	539	442	449
Poly-3 test	P=0.005	P=0.206	P=0.010	P=0.006
Hepatoblastoma, Multiple	0	0	2	0
Hepatoblastoma (includes multiple)[j]				
Overall rate	1/50 (2%)	5/50 (10%)	10/50 (20%)	8/50 (16%)
Adjusted rate	2.3%	10.8%	22.3%	17.3%
Terminal rate	1/34 (3%)	3/36 (8%)	4/31 (13%)	3/36 (8%)
First incidence (days)	730 (T)	539	512	580
Poly-3 test	P=0.064	P=0.121	P=0.005	P=0.021
Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma[k]				
Overall rate	38/50 (76%)	45/50 (90%)	48/50 (96%)	48/50 (96%)
Adjusted rate	83.1%	91.7%	99.2%	98.6%
Terminal rate	28/34 (82%)	34/36 (94%)	31/31 (100%)	36/36 (100%)
First incidence (days)	548	539	442	449
Poly-3 test	P=0.006	P=0.157	P=0.004	P=0.006

TABLE 19
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Female				
Number Examined Microscopically	50	50	50	50
Clear Cell Focus	0	2	2	3
Eosinophilic Focus	20	18	45**	38**
Mixed Cell Focus	3	9	7	7
Fatty Change	1 (4.0)	0	0	8** (2.5)
Hepatocyte, Hypertrophy	0	11** (1.6)	10** (1.6)	17** (1.9)
Necrosis	1 (2.0)	8* (1.5)	4 (2.0)	10** (1.8)
Hepatocellular Adenoma, Multiple	2	6	29**	35**
Hepatocellular Adenoma (includes multiple)[l]				
Overall rate	17/50 (34%)	19/50 (38%)	37/50 (74%)	44/50 (88%)
Adjusted rate	35.5%	41.1%	80.0%	96.1%
Terminal rate	16/43 (37%)	17/40 (43%)	34/39 (87%)	31/32 (97%)
First incidence (days)	698	720	649	481
Poly-3 test	P<0.001	P=0.364	P<0.001	P<0.001
Hepatocellular Carcinoma, Multiple	1	3	5	19**
Hepatocellular Carcinoma (includes multiple)[m]				
Overall rate	6/50 (12%)	13/50 (26%)	18/50 (36%)	31/50 (62%)
Adjusted rate	12.5%	28.2%	39.3%	71.9%
Terminal rate	4/43 (9%)	12/40 (30%)	16/39 (41%)	24/32 (75%)
First incidence (days)	666	720	669	512
Poly-3 test	P<0.001	P=0.049	P=0.002	P<0.001
Hepatocellular Adenoma or Carcinoma[n]				
Overall rate	20/50 (40%)	25/50 (50%)	42/50 (84%)	45/50 (90%)
Adjusted rate	41.6%	54.1%	90.6%	98.3%
Terminal rate	18/43 (42%)	23/40 (58%)	38/39 (97%)	32/32 (100%)
First incidence (days)	666	720	649	481
Poly-3 test	P<0.001	P=0.154	P<0.001	P<0.001
Hepatoblastoma[o]				
Overall rate	0/50 (0%)	1/50 (2%)	0/50 (0%)	4/50 (8%)
Adjusted rate	0.0%	2.2%	0.0%	9.7%
Terminal rate	0/43 (0%)	1/40 (3%)	0/39 (0%)	3/32 (9%)
First incidence (days)	—[p]	729 (T)	—	699
Poly-3 test	P=0.007	P=0.493	—[q]	P=0.044

TABLE 19
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Female (continued)				
Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma[r]				
Overall rate	20/50 (40%)	26/50 (52%)	42/50 (84%)	45/50 (90%)
Adjusted rate	41.6%	56.3%	90.6%	98.3%
Terminal rate	18/43 (42%)	24/40 (60%)	38/39 (97%)	32/32 (100%)
First incidence (days)	666	720	649	481
Poly-3 test	P<0.001	P=0.108	P<0.001	P<0.001

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
(T) Terminal kill
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year gavage studies with corn oil vehicle controls (mean ± standard deviation): 181/350 (51.7% ± 6.9%), range 44%-62%; all routes: 658/1,149 (57.3% ± 12.6%), range 24%-78%
[d] Historical incidence for corn oil gavage studies: 116/350 (33.1% ± 10.5%), range 16%-44%; all routes: 399/1,149 (34.7% ± 10.8%), range 16%-56%
[e] Number of animals with neoplasm per number of animals with liver examined microscopically
[f] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[g] Observed incidence at terminal kill
[h] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
[i] Historical incidence for corn oil gavage studies: 239/350 (68.3% ± 8.9%), range 56%-78%; all routes: 844/1,149 (73.5% ± 11.3%), range 52%-90%
[j] Historical incidence for corn oil gavage studies: 14/350 (4.0% ± 2.8%), range 0%-8%; all routes: 61/1,149 (5.3% ± 7.1%), range 0%-34%
[k] Historical incidence for corn oil gavage studies: 242/350 (69.1% ± 8.0%), range 58%-78%; all routes: 852/1,149 (74.2% ± 11.5%), range 52%-92%
[l] Historical incidence for corn oil gavage studies: 75/347 (21.6% ± 10.8%), range 6%-34%; all routes: 380/1,195 (31.8% ± 21.4%), range 2%-78%
[m] Historical incidence for corn oil gavage studies: 29/347 (8.3% ± 5.5%), range 2%-18%; all routes: 144/1,195 (12.1% ± 10.8%), range 0%-46%
[n] Historical incidence for corn oil gavage studies: 91/347 (26.2% ± 12.7%), range 8%-40%; all routes: 444/1,195 (37.2% ± 22.9%), range 6%-82%
[o] Historical incidence for corn oil gavage studies: 1/347 (0.3% ± 0.8%), range 0%-2%; all routes: 4/1,195 (0.3% ± 0.8%), range 0%-2%
[p] Not applicable; no neoplasm in animal group
[q] Value of statistic cannot be computed.
[r] Historical incidence for corn oil gavage studies: 91/347 (26.2% ± 12.7%), range 8%-40%; all routes: 444/1,195 (37.2% ± 22.9%), range 6%-82%

involved larger areas of hepatic parenchyma. Necrosis was not recorded as a separate lesion if it occurred within a neoplasm.

Lung: There were significantly increased incidences of alveolar/bronchiolar adenoma and adenoma or carcinoma (combined) in 20 and 60 mg/kg females (Tables 20, D1, and D2). In females, the incidences of alveolar/bronchiolar adenoma in the 20 and 60 mg/kg groups exceeded the historical control ranges for corn oil gavage studies and for all routes of exposure (Tables 20 and D3b). In male mice, the incidences of alveolar/bronchiolar adenoma, carcinoma, and adenoma or carcinoma (combined) were not significantly increased (Tables 20 and C2), but the incidences of alveolar/bronchiolar adenoma in the 6 and 20 mg/kg groups exceeded the historical control ranges for corn oil gavage studies and for all routes of exposure (Tables 20 and C3b). However, in male mice, the lack of statistical significance or a dose response, as well as the incidences of adenoma or carcinoma (combined) falling within the historical control range, led to the conclusion that alveolar/bronchiolar neoplasms in male mice were not related to *N,N*-dimethyl-*p*-toluidine exposure. Alveolar/bronchiolar adenomas were discrete lesions that caused compression of the surrounding lung

TABLE 20
Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Male				
Number Examined Microscopically	50	50	50	50
Alveolus, Infiltration Cellular, Histiocyte[a]	1 (2.0)[b]	2 (1.5)	2 (2.5)	10** (1.2)
Alveolar/bronchiolar Adenoma, Multiple	0	5	1	0
Alveolar/bronchiolar Adenoma (includes multiple)[c]	11	16	18	10
Alveolar/bronchiolar Carcinoma, Multiple	0	1	0	0
Alveolar/bronchiolar Carcinoma (includes multiple)[d]	2	3	0	4
Alveolar/bronchiolar Adenoma or Carcinoma[e]				
Overall rate[f]	13/50 (26%)	19/50 (38%)	18/50 (36%)	12/50 (24%)
Adjusted rate[g]	30.0%	41.2%	41.1%	26.2%
Terminal rate[h]	11/34 (32%)	16/36 (44%)	13/31 (42%)	8/36 (22%)
First incidence (days)	643	568	609	562
Poly-3 test[i]	P=0.167N	P=0.187	P=0.194	P=0.433N
Female				
Number Examined Microscopically	50	50	50	50
Alveolar Epithelium, Hyperplasia	2 (3.0)	3 (2.3)	8* (1.5)	2 (1.0)
Alveolus, Infiltration Cellular, Histiocyte	1 (1.0)	0	0	7* (1.4)
Bronchiole, Epithelium, Regeneration	0	0	0	5* (1.8)
Bronchus, Epithelium, Regeneration	0	0	0	5* (2.0)
Bronchus, Necrosis	0	0	0	5* (1.6)
Alveolar/bronchiolar Adenoma, Multiple	2	1	1	0
Alveolar/bronchiolar Adenoma (includes multiple)[j]				
Overall rate	2/50 (4%)	4/50 (8%)	8/50 (16%)	12/50 (24%)
Adjusted rate	4.2%	8.7%	17.5%	28.2%
Terminal rate	2/43 (5%)	4/40 (10%)	7/39 (18%)	8/32 (25%)
First incidence (days)	729 (T)	729 (T)	649	570
Poly-3 test	P<0.001	P=0.322	P=0.039	P<0.001
Alveolar/bronchiolar Carcinoma[k]	0	1	2	1

TABLE 20 (continued)
Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Female (continued)				
Alveolar/bronchiolar Adenoma or Carcinoma[l]				
Overall rate	2/50 (4%)	5/50 (10%)	9/50 (18%)	13/50 (26%)
Adjusted rate	4.2%	10.8%	19.6%	30.3%
Terminal rate	2/43 (5%)	5/40 (13%)	7/39 (18%)	8/32 (25%)
First incidence (days)	729 (T)	729 (T)	649	570
Poly-3 test	P<0.001	P=0.203	P=0.021	P<0.001

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
(T) Terminal kill
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year gavage studies with corn oil vehicle controls (mean ± standard deviation): 53/350 (15.1% ± 4.1%), range 10%-22%; all routes: 172/1,150 (15.0% ± 6.9%), range 2%-30%
[d] Historical incidence for corn oil gavage studies: 28/350 (8.0% ± 6.5%), range 4%-22%; all routes: 144/1,150 (12.5% ± 7.1%), range 4%-24%
[e] Historical incidence for corn oil gavage studies: 77/350 (22.0% ± 7.3%), range 14%-34%; all routes: 301/1,150 (26.2% ± 6.3%), range 14%-40%
[f] Number of animals with neoplasm per number of animals with lung examined microscopically
[g] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[h] Observed incidence at terminal kill
[i] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in a dose group is indicated by **N**.
[j] Historical incidence for corn oil gavage studies: 16/346 (4.6% ± 3.1%), range 0%-8%; all routes: 60/1,196 (5.0% ± 3.6%), range 0%-12%
[k] Historical incidence for corn oil gavage studies: 7/346 (2.0% ± 2.0%), range 0%-4%; all routes: 44/1,196 (3.7% ± 3.3%), range 0%-14%
[l] Historical incidence for corn oil gavage studies: 23/346 (6.7% ± 3.

parenchyma (Plates 30 and 31). Most commonly, they had a papillary pattern and consisted of projections of epithelial cells on a fine fibrovascular stalk. The epithelial cells tended to be uniform, in contrast to the cells in carcinomas, which displayed anisocytosis and anisokaryosis, and often areas of piling. Carcinomas also tended to be larger and more invasive lesions than adenomas and were more likely to have different growth patterns, such as solid and papillary, within a single tumor.

In 20 mg/kg females, the incidence of alveolar epithelium hyperplasia was significantly greater than that in the vehicle controls (Tables 20 and D4). Incidences of necrosis of the bronchus and regeneration of the bronchial and bronchiolar epithelium were significantly increased in 60 mg/kg females; these lesions did not occur in vehicle control females. There were significantly increased incidences of histiocytic infiltrates in the alveoli of 60 mg/kg males and females. Alveolar epithelial hyperplasia was characterized by focal but poorly demarcated areas in which increased type II pneumocytes lined the alveolar septae. Necrosis of the bronchus was characterized by areas where epithelial cells were overtly necrotic (often detaching and hypereosinophilic with karyorrhectic nuclei), while epithelial regeneration was characterized by areas where epithelial cells were cuboidal or attenuated and basophilic, rather than columnar and eosinophilic. Alveolar infiltrates of histiocytes consisted of collections of medium to large cells with foamy cytoplasm within the alveolar spaces; this lesion was not recorded when cellular infiltrates were associated with a neoplasm.

Forestomach: There were significantly increased incidences of squamous cell papilloma and squamous cell papilloma or carcinoma (combined) in 20 and 60 mg/kg females (Tables 21, D1, and D2) and these incidences exceeded the historical control ranges for corn oil gavage studies and for all routes of exposure (Tables 21 and D3c). Squamous cell carcinoma occurred in one 6 mg/kg female. In addition, there were significantly

TABLE 21
Incidences of Neoplasms and Nonneoplastic Lesions of the Forestomach in Female Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Number Necropsied	50	50	50	50
Epithelium, Hyperplasia[a]	3 (2.7)[b]	5 (2.8)	12** (2.2)	17** (2.6)
Inflammation	3 (3.0)	4 (2.0)	7 (2.3)	16** (2.3)
Ulcer	2 (2.0)	2 (2.0)	4 (1.3)	7* (1.6)
Squamous Cell Papilloma, Multiple	0	1	1	0
Squamous Cell Papilloma (includes multiple)[c]				
Overall rate[d]	1/50 (2%)	5/50 (10%)	6/50 (12%)	7/50 (14%)
Adjusted rate[e]	2.1%	10.8%	13.2%	17.1%
Terminal rate[f]	1/43 (2%)	5/40 (13%)	5/39 (13%)	6/32 (19%)
First incidence (days)	729 (T)	729 (T)	703	708
Poly-3 test[g]	P=0.037	P=0.094	P=0.049	P=0.017
Squamous Cell Carcinoma[h]	0	1	0	0
Squamous Cell Papilloma or Carcinoma[i]				
Overall rate	1/50 (2%)	6/50 (12%)	6/50 (12%)	7/50 (14%)
Adjusted rate	2.1%	13.0%	13.2%	17.1%
Terminal rate	1/43 (2%)	6/40 (15%)	5/39 (13%)	6/32 (19%)
First incidence (days)	729 (T)	729 (T)	703	708
Poly-3 test	P=0.055	P=0.051	P=0.049	P=0.017

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
(T) Terminal kill
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year gavage studies with corn oil vehicle controls (mean ± standard deviation): 12/348 (3.5% ± 1.5%), range 2%-6%; all routes: 22/1,198 (1.8% ± 1.7%), range 0%-6%
[d] Number of animals with neoplasm per number of animals necropsied
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
[h] Historical incidence for corn oil gavage studies: 0/348; all routes: 1/1,198 (0.1% ± 0.4%), range 0%-2%
[i] Historical incidence for corn oil gavage studies: 12/348 (3.5% ± 1.5%), range 2%-6%; all routes: 23/1,198 (1.9% ± 1.6%), range 0%-6%

increased incidences of epithelial hyperplasia in 20 and 60 mg/kg females and inflammation and ulcer in 60 mg/kg females (Tables 21 and D4). Squamous cell papillomas were typical, exophytic lesions (Plate 32). In contrast to carcinomas, benign neoplasms lack any disruption of the basement membrane and have no invasion into the underlying lamina propria. Epithelial hyperplasia was characterized by focal or focally extensive areas of thickening of the squamous epithelial layer of the forestomach (Plate 33). Ulcers were characterized by full thickness necrosis of the epithelium. Inflammation, composed of a mixture of cell types, was usually associated with erosion, ulceration, or hyperplasia.

Nose and Olfactory lobe: One adenoma of the respiratory epithelium of the nose occurred in a 6 mg/kg male (Table C1). While this incidence was not statistically significant, there are no adenomas or carcinomas of the respiratory epithelium of the nose in the historical control data. The adenoma was composed of proliferations of papillary projections and infoldings lined by cuboidal to columnar, pseudostratified, ciliated epithelium.

In the respiratory epithelium of 60 mg/kg females, the incidences of hyperplasia and necrosis were significantly greater than those of the vehicle controls (Tables 22 and D4). In the glands underlying the respiratory epithelium, there were significantly increased

TABLE 22
Incidences of Nonneoplastic Lesions of the Nose and Brain in Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control		6 mg/kg		20 mg/kg		60 mg/kg	
Male								
Nose[a]	49		50		50		50	
Glands, Olfactory Epithelium, Dilatation[b]	4	(1.0)[c]	11	(1.0)	7	(1.0)	48**	(1.8)
Glands, Olfactory Epithelium, Hyperplasia	4	(1.0)	9	(1.1)	7	(1.3)	49**	(2.1)
Glands, Olfactory Epithelium, Metaplasia, Respiratory	5	(1.0)	5	(1.0)	6	(1.0)	48**	(1.7)
Glands, Respiratory Epithelium, Dilatation	17	(1.0)	19	(1.0)	13	(1.0)	41**	(1.8)
Glands, Respiratory Epithelium, Hyperplasia	4	(1.0)	2	(1.0)	2	(1.0)	11	(1.1)
Glands, Respiratory Epithelium, Metaplasia, Respiratory	2	(1.5)	2	(1.0)	2	(1.0)	10*	(1.1)
Nasolacrimal Duct, Hyperplasia, Regenerative	0		0		0		4	(1.0)
Nerve, Atrophy	2	(1.0)	7	(1.1)	4	(1.3)	42**	(2.0)
Olfactory Epithelium, Metaplasia, Respiratory	10	(1.3)	10	(1.3)	5	(1.2)	49**	(2.3)
Olfactory Epithelium, Necrosis	1	(1.0)	3	(1.3)	3	(1.0)	8*	(1.5)
Vomeronasal Organ, Necrosis	0		1	(2.0)	2	(1.0)	3	(1.0)
Olfactory Lobe	38		43		39		34	
Atrophy	0		1	(3.0)	0		5*	(1.2)
Female								
Nose	50		49		50		50	
Glands, Olfactory Epithelium, Dilatation	13	(1.0)	14	(1.1)	20	(1.0)	46**	(2.3)
Glands, Olfactory Epithelium, Hyperplasia	2	(1.0)	14**	(1.0)	14**	(1.1)	50**	(2.2)
Glands, Olfactory Epithelium, Metaplasia, Respiratory	2	(1.0)	5	(1.0)	7	(1.0)	44**	(2.3)
Glands, Respiratory Epithelium, Dilatation	10	(1.0)	17	(1.0)	15	(1.1)	33**	(1.4)
Glands, Respiratory Epithelium, Hyperplasia	0		2	(1.0)	12**	(1.2)	13**	(1.2)
Glands, Respiratory Epithelium, Metaplasia, Respiratory	0		0		10**	(1.0)	10**	(1.4)
Inflammation	3	(1.0)	7	(1.0)	3	(1.0)	32**	(1.3)
Nasolacrimal Duct, Hyperplasia, Regenerative	0		0		0		4*	(2.5)
Nerve, Atrophy	0		0		0		41**	(2.3)
Olfactory Epithelium, Accumulation, Hyaline Droplet	2	(1.0)	5	(1.0)	8*	(1.0)	15**	(1.1)
Olfactory Epithelium, Metaplasia, Respiratory	1	(1.0)	6*	(1.0)	14**	(1.1)	46**	(2.9)
Olfactory Epithelium, Necrosis	0		0		3	(1.3)	6*	(2.3)
Respiratory Epithelium, Hyperplasia	11	(1.0)	15	(1.0)	11	(1.0)	30**	(1.2)
Respiratory Epithelium, Necrosis	0		0		0		5*	(2.0)
Vomeronasal Organ, Necrosis	0		0		0		4*	(1.5)
Olfactory Lobe	27		34		24		29	
Atrophy	0		0		0		8**	(1.6)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

incidences of dilatation in 60 mg/kg males and females, hyperplasia in 20 and 60 mg/kg females, and respiratory metaplasia in 20 mg/kg females and 60 mg/kg males and females (Tables 22, C4, and D4). Hyperplasia was also increased in 60 mg/kg males, but the increase was not significant. Hyperplasia of the respiratory epithelium consisted of increased numbers of cells in the respiratory epithelium, with resultant nuclear crowding and infoldings of epithelium. Necrosis of the respiratory epithelium was characterized by widespread necrosis of the epithelium throughout the nasal section; it was usually seen in conjunction with necrosis of the transitional and olfactory epithelium. In the glands underlying the respiratory epithelium it was often difficult to tell hyperplastic glands that had undergone respiratory metaplasia from hyperplasia of the respiratory epithelium that produced infoldings into the underlying lamina propria (pseudogland formation). Hyperplasia of the glands was characterized by increased profiles of glands in the lamina propria underlying the respiratory epithelium; these glands were often lined by increased numbers of cells, which were crowded together and often metaplastic. Dilated glands had enlarged luminal areas, and often contained amorphous, eosinophilic material, inflammatory cells, or cell debris. Respiratory metaplasia of the glands was characterized by glands lined by cuboidal to columnar ciliated epithelial cells.

In the olfactory epithelium of the nose, there were significantly increased incidences of respiratory metaplasia in 60 mg/kg males and all dosed female groups (Tables 22, C4, and D4). There were also significantly increased incidences of necrosis in 60 mg/kg males and females and hyaline droplet accumulation in 20 and 60 mg/kg females. In males, there was a negative trend in the incidence of hyaline droplet accumulation (Table C4). In the glands underlying the olfactory epithelium, there were significantly increased incidences of dilatation and respiratory metaplasia in 60 mg/kg males and females and hyperplasia in 60 mg/kg males and all dosed female groups.

Respiratory metaplasia of the olfactory epithelium was diagnosed when the normal olfactory epithelium was replaced with cuboidal to columnar pseudostratified ciliated epithelium. Necrosis of the olfactory epithelium was characterized by individual cell necrosis within the layer of olfactory epithelium in some animals. Typically, there were decreased numbers of cells in the olfactory epithelium of affected animals. In other animals, there was denudement of the olfactory epithelium as part of widespread necrosis of the epithelial layers in the nose. Hyaline droplet accumulation consisted of brightly eosinophilic droplets or globules within the cytoplasm of epithelial cells. Hyperplasia of the glands underlying the olfactory epithelium was diagnosed when there were increased profiles of glands and/or the glands were composed of increased numbers of cells (Plates 34 and 35). Respiratory metaplasia of these glands was diagnosed when the epithelium of the glands consisted of pseudostratified, ciliated, cuboidal to columnar epithelium (Plate 36). Dilated glands had enlarged luminal areas, and often contained amorphous, eosinophilic material, inflammatory cells, or cell debris.

In 60 mg/kg females, there were significantly increased incidences of regenerative hyperplasia of the nasolacrimal duct, necrosis of the vomeronasal organ, and inflammation of the nose; these lesions were also increased in 60 mg/kg males, but not significantly (Tables 22, C4, and D4). In 60 mg/kg males and females, there were significantly increased incidences of nerve atrophy in the nose and olfactory lobe atrophy in the brain. A review of the nasal sections of the brain indicated that a portion of the olfactory lobe was visible on the most caudal section of the nasal cavity in many, but not all, mice (males: vehicle control, 38/50; 6 mg/kg, 43/50; 20 mg/kg, 39/50; 60 mg/kg, 34/50; females: 27/50, 34/50, 24/50, 29/49). Because the olfactory lobe is part of the brain, these diagnoses were recorded in the Tables C4 and D4 under the category of brain. While sections of the brain were examined for all animals, the denominators for olfactory lobes examined presented in Table 22 are the number observed on the nasal sections of the brain. Regenerative hyperplasia of the nasolacrimal duct was characterized by attenuated epithelial cells containing large vesicular nuclei and displaying cellular pleomorphism. Necrosis of the vomeronasal organ was characterized by loss of cellular detail, nuclear pyknosis, and replacement of columnar epithelium by cell debris. Inflammation of the nose consisted predominantly of neutrophils with fewer macrophages, and occasional plasma cells and eosinophils in the lamina propria of the olfactory, respiratory, and/or transitional epithelial regions, and within the nasal cavity. Nerve atrophy was characterized by a decrease in the numbers of nerves, with remaining nerves being vacuolated and/or irregular in outline. Atrophy of the olfactory lobe of the brain was characterized by the shrinkage and vacuolation of the neuropil.

Hematopoietic System (Spleen, Bone Marrow, and Mandibular and Mesenteric Lymph Nodes): In the spleen, there were increased incidences of atrophy in 20 and 60 mg/kg males, but only the incidence in the 20 mg/kg group was statistically significant (Tables 23 and C4).

TABLE 23
Incidences of Nonneoplastic Lesions of the Hematopoietic System in Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Male				
Spleen[a]	48	50	49	50
Atrophy[b]	4 (2.5)[c]	11 (2.2)	11* (2.4)	6 (1.8)
Female				
Bone Marrow	50	50	50	49
Hyperplasia	5 (2.2)	14* (1.9)	15** (2.1)	14** (2.1)
Lymph Node, Mesenteric	49	49	49	50
Atrophy	1 (2.0)	5 (2.0)	5 (2.2)	12** (2.9)
Hyperplasia, Lymphoid	7 (2.3)	3 (3.7)	1* (2.0)	0*
Spleen	49	49	49	50
Red Pulp, Atrophy	0	0	0	5* (3.2)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

The incidence of red pulp atrophy in 60 mg/kg females was significantly increased compared to the vehicle controls (Tables 23 and D4). Atrophy of the spleen was diagnosed when there was a decrease in the size of lymphoid follicles when compared to the vehicle controls, and atrophy of the red pulp was diagnosed when there was a reduction in the amount of red pulp, with compressed vascular spaces.

In bone marrow, the incidences of hyperplasia were significantly increased in all dosed female groups (Tables 23 and D4). Bone marrow hyperplasia was diagnosed when there was an increase in cellularity of any myeloid, erythroid, or megakaryocytic lineages, though generally the erythroid and myeloid series were the predominant cell types present.

In the mesenteric lymph node, the incidences of atrophy were significantly increased in 60 mg/kg females (Tables 23 and D4). There was also a negative trend in the incidences of lymphoid hyperplasia of the mesenteric lymph node in females. Atrophy of the lymph node was based upon reduction in lymphocyte density in the cortex and paracortex and in reduction in overall lymph node size. Lymphoid hyperplasia was characterized by an increased number of follicles, and/or an increase in cellularity, due to increased numbers of lymphocytes, in the section of lymph node.

Harderian Gland: There were significantly decreased incidences of adenoma or carcinoma (combined) in 20 and 60 mg/kg males (11/50, 8/50, 5/50, 2/50; Tables C1 and C2). The incidence of Harderian gland adenoma or carcinoma (combined) in 60 mg/kg males is below the historical control ranges for corn oil gavage studies [57/350 (16% ± 5%), range 10% to 24%] and all routes of exposure [184/1,150 (16% ± 5%), range 6% to 24%]. It is uncertain if these decreased incidences were related to treatment.

GENETIC TOXICOLOGY

N,N-Dimethyl-*p*-toluidine was tested in two independent bacterial gene mutation studies, and negative results were obtained in both studies (Tables E1 and E2). In the first study (concentration range, 0.33 to 1,000 µg/plate), no increases in the numbers of mutant colonies were seen in *Salmonella typhimurium* strains TA97, TA98, TA100, or TA1535, with or without 10% or 30% S9 derived from induced hamster or rat liver. In the second study, which tested the same chemical lot

(050404) that was used in the 2-year studies, negative results were obtained over a concentration range of 50 to 1,500 µg/plate in *S. typhimurium* strains TA98 and TA100 and in *Escherichia coli* WP2 *uvrA*/pKM101, with and without 10% rat liver S9.

In vivo, no significant increases in the frequencies of micronucleated erythrocytes, an indicator of chromosomal damage, were observed in peripheral blood of male or female B6C3F1/N mice treated with 15 to 125 mg/kg per day *N*,*N*-dimethyl-*p*-toluidine by gavage for 3 months (Table E3). No significant alterations in the percentage of circulating polychromatic erythrocytes (reticulocytes) were observed, suggesting that *N*,*N*-dimethyl-*p*-toluidine did not induce bone marrow toxicity over the dose range tested. Results of a second micronucleus test in male B6C3F1/N mice administered 30 to 75 mg/kg *N*,*N*-dimethyl-*p*-toluidine (lot 050404) by gavage once daily for 4 days were also negative and again, no significant alterations in the percentage of circulating reticulocytes were observed (Table E4).

Two independent comet assays were conducted with *N*,*N*-dimethyl-*p*-toluidine to measure induction of DNA damage in liver and blood leukocytes. In the first study, conducted in male B6C3F1/N mice, *N*,*N*-dimethyl-*p*-toluidine administered by gavage over a range of 30 to 75 mg/kg once daily for 4 days did not produce an increase in DNA migration in liver cells or blood leukocytes (Table E5). In the second study, conducted in male Sprague-Dawley rats, *N*,*N*-dimethyl-*p*-toluidine administered by gavage at a single dose of 60 mg/kg per day for 4 days was associated with a small but statistically significant increase in percent tail DNA in liver cells compared with the vehicle control group (Table E6).

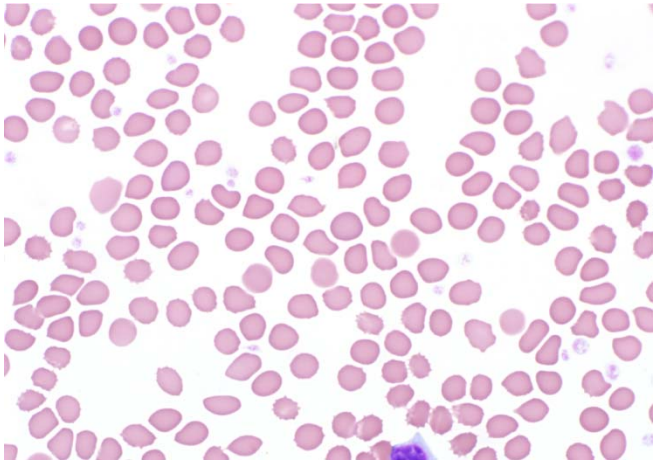


PLATE 1
Blood smear on day 25 from a vehicle control female F344/N rat in the 3-month gavage study of *N*,*N*-dimethyl-*p*-toluidine.

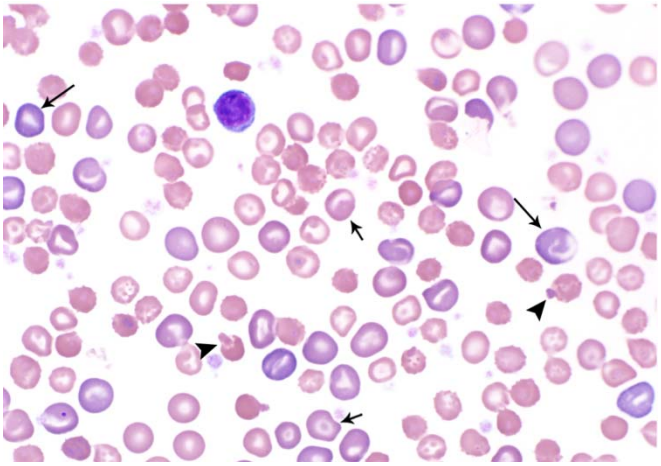


PLATE 2
Blood smear on day 25 from a female F344/N rat administered 500 mg/kg *N*,*N*-dimethyl-*p*-toluidine by gavage for 3 months. Note the increased central pallor of erythrocytes, increased numbers of polychromatophilic (long arrows) and leptocytic (short arrows) erythrocytes, and Heinz bodies (arrowheads). Leptocytes included various forms of stomatocytes, knizocytes, and codocytes; the majority was polychromatophilic and is consistent with responsive anemia.

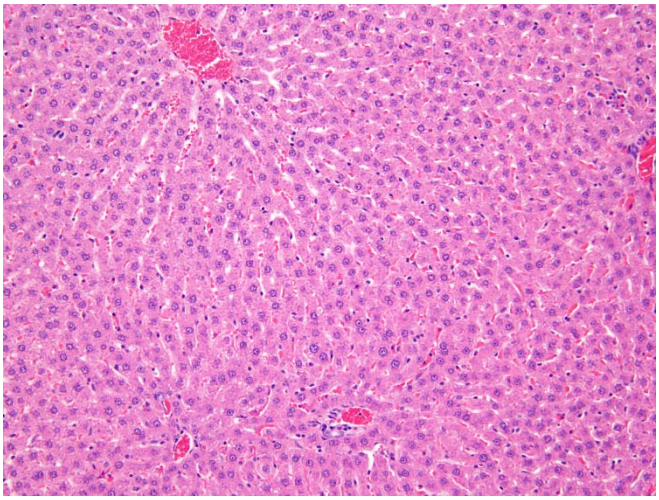


PLATE 3
Liver of a vehicle control female F344/N rat in the 3-month gavage study of *N*,*N*-dimethyl-*p*-toluidine. H&E

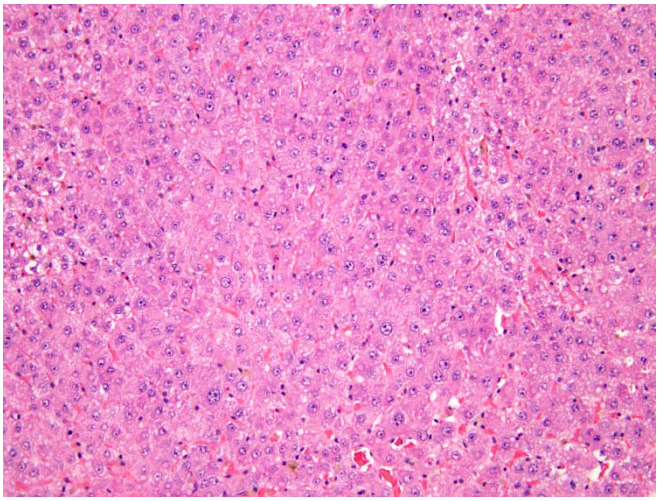


PLATE 4
Moderate hepatocellular hypertrophy in the liver of a female F344/N rat administered 500 mg/kg *N*,*N*-dimethyl-*p*-toluidine by gavage for 3 months. H&E

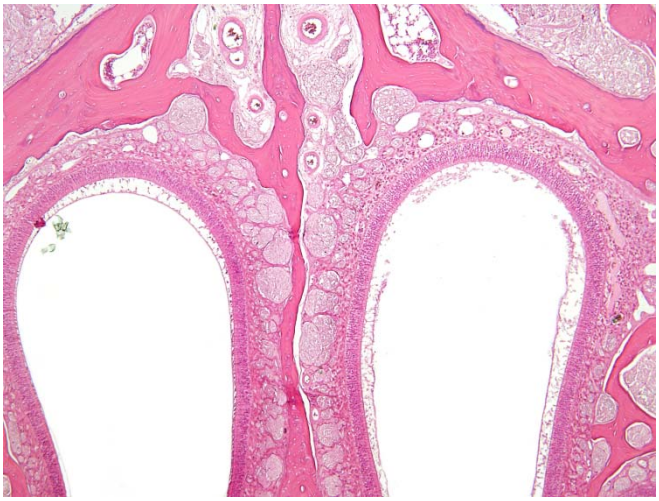


PLATE 5
Level III nasal section of a vehicle control female F344/N rat in the 3-month gavage study of *N*,*N*-dimethyl-*p*-toluidine. H&E

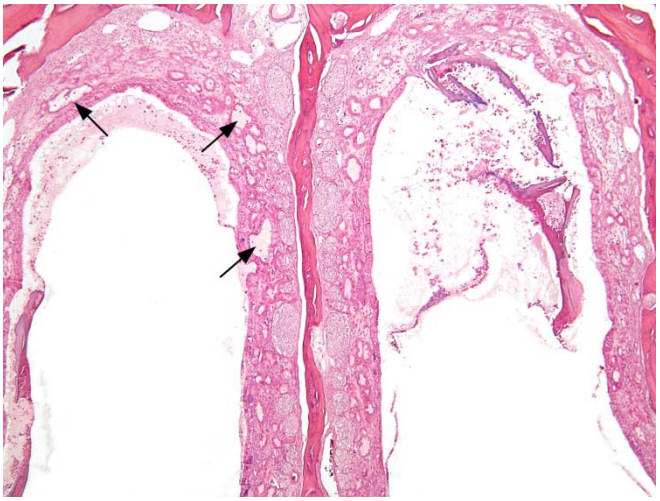


PLATE 6
Level III nasal section showing degeneration of the olfactory epithelium and dilatation (arrows) and hyperplasia of the underlying glands in a male F344/N rat administered 500 mg/kg *N*,*N*-dimethyl-*p*-toluidine by gavage for 3 months. H&E

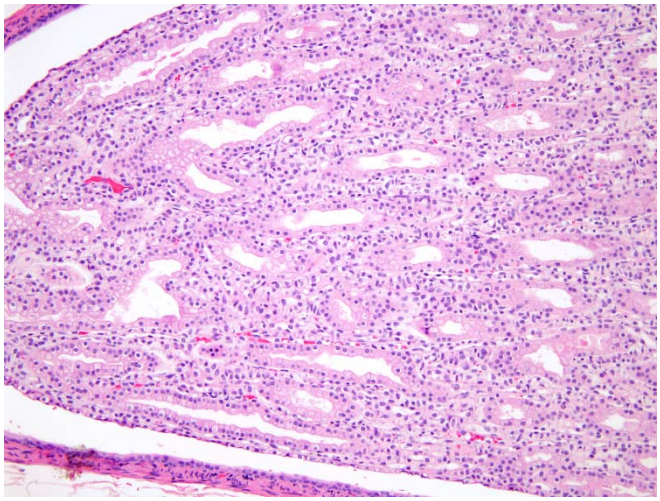


PLATE 7
Section of the renal papilla of a vehicle control male F344/N rat in the 3-month gavage study of *N*,*N*-dimethyl-*p*-toluidine. H&E

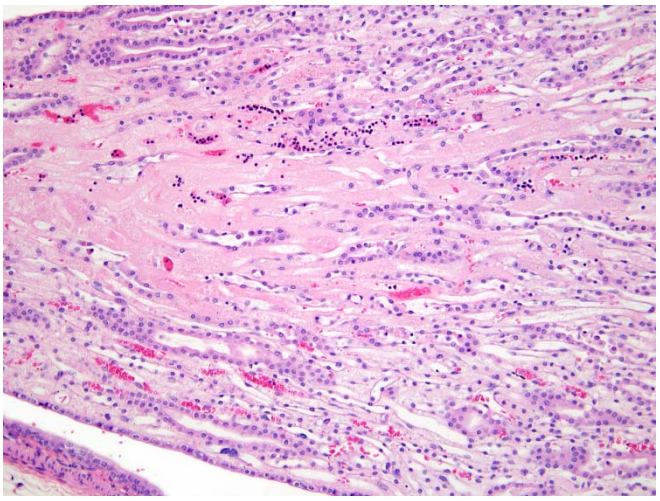


PLATE 8
Widespread necrosis in the renal papilla of a male F344/N rat administered 500 mg/kg *N*,*N*-dimethyl-*p*-toluidine by gavage for 3 months. H&E

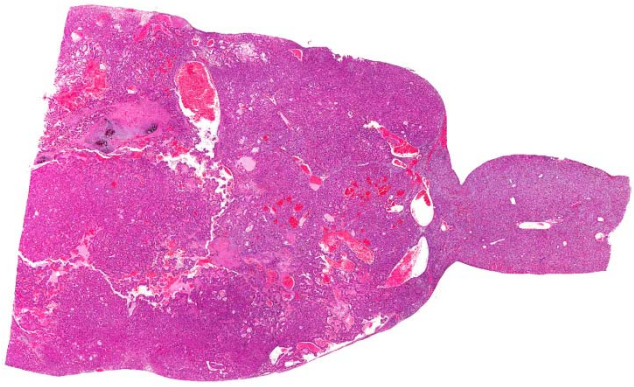


PLATE 9
Hepatocellular carcinoma in the liver of a male F344/N rat administered 60 mg/kg *N*,*N*-dimethyl-*p*-toluidine by gavage for 2 years. H&E

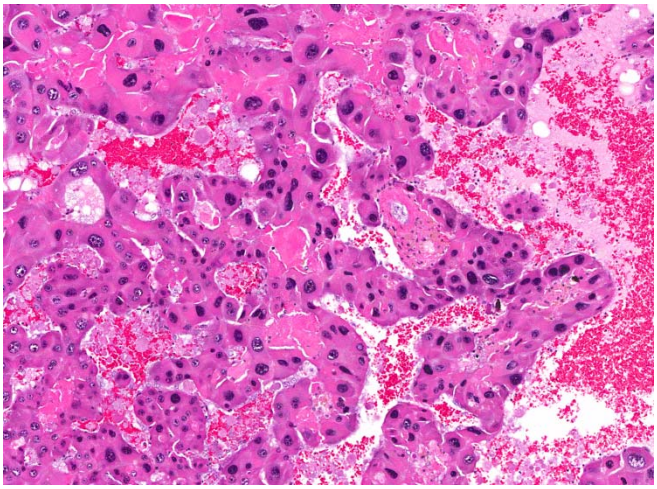


PLATE 10
Higher magnification of the hepatocellular carcinoma in Plate 9. Note the thickened, irregular trabeculae with loss of the normal hepatic architecture and cells displaying pleomorphism and anisokaryosis. H&E

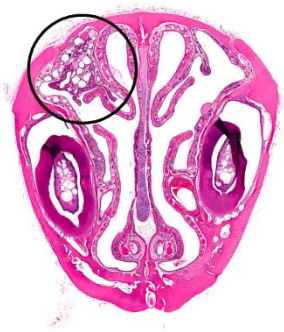


PLATE 11
Transitional epithelial adenoma (circled area) on the lateral wall in Level I of the nose in a male F344/N rat administered 60 mg/kg *N*,*N*-dimethyl-*p*-toluidine by gavage for 2 years. H&E

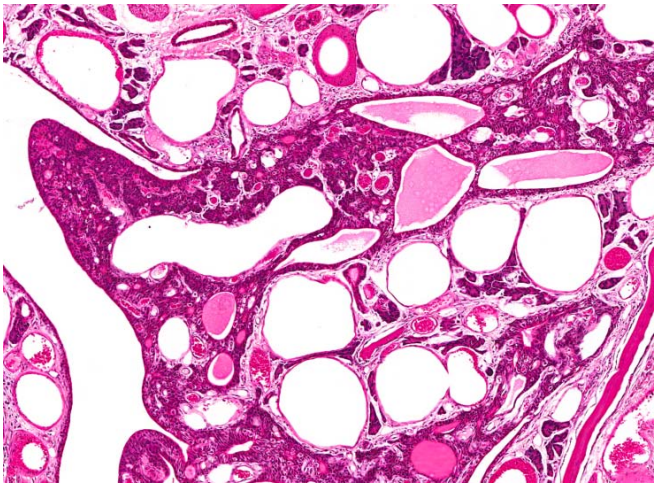


PLATE 12
Higher magnification of the transitional epithelial adenoma in Plate 11. Note that, in contrast to the carcinoma (Plates 13, 14, and 15), there is no invasion into the underlying tissue and the cells are more differentiated. H&E

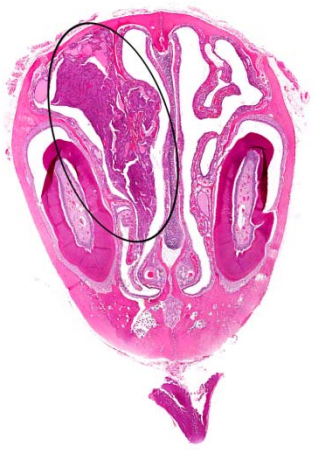


PLATE 13
Transitional epithelial carcinoma (circled area) arising in the dorsolateral aspect of the nasal cavity in nasal Level I in a male F344/N rat administered 60 mg/kg *N*,*N*-dimethyl-*p*-toluidine by gavage for 2 years. H&E

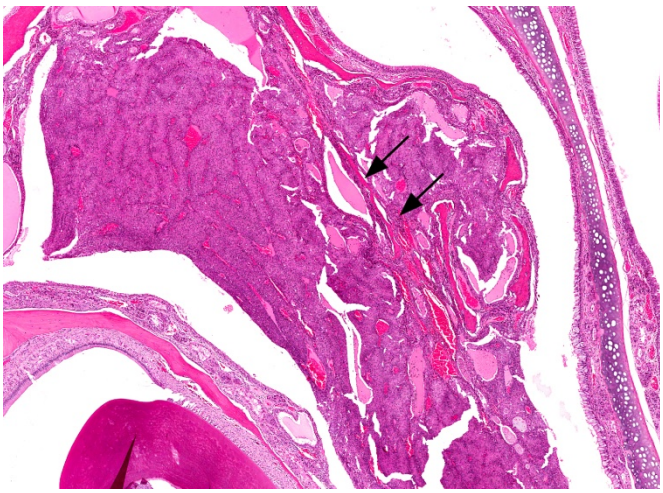


PLATE 14
Higher magnification of the transitional epithelial carcinoma in Plate 13. Note invasion through the bone of the turbinate (arrows). H&E

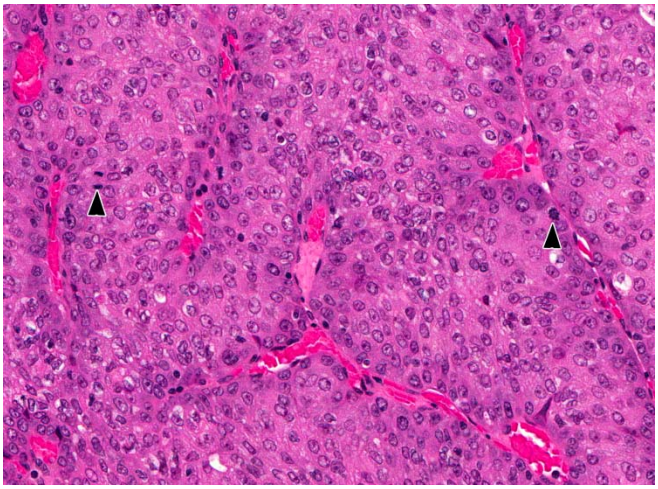


PLATE 15
Higher magnification of the transitional epithelial carcinoma in Plate 14. Note cells in division (mitotic figures) (arrowheads). H&E

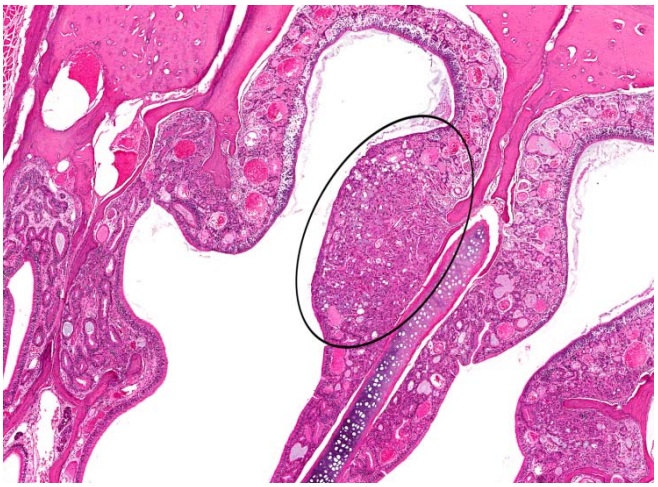


PLATE 16
Nasal adenoma arising from the glands underlying the olfactory epithelium (circled area) in nasal Level II in a male F344/N rat administered 60 mg/kg *N*,*N*-dimethyl-*p*-toluidine by gavage for 2 years. H&E

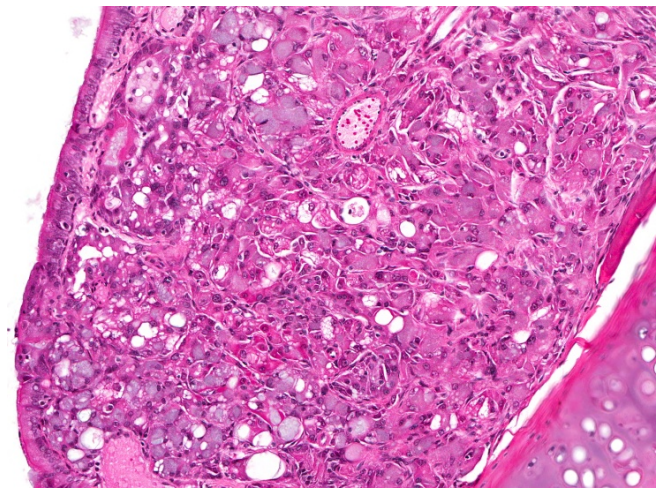


PLATE 17
Higher magnification of the adenoma in Plate 16. Note the mixed pattern of cells. H&E

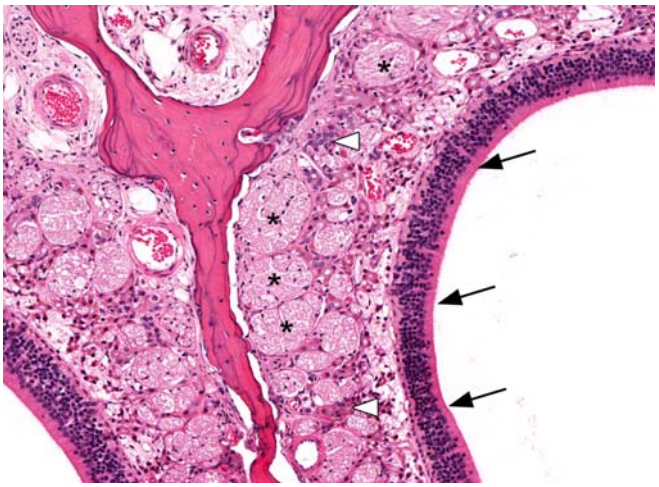


PLATE 18
Normal olfactory epithelium (arrows) in a vehicle control male F344/N rat in the 2-year gavage study of *N*,*N*-dimethyl-*p*-toluidine. Note the orderly arrangement. The lamina propria contains numerous nerve bundles (asterisks) and glands (arrowheads). H&E

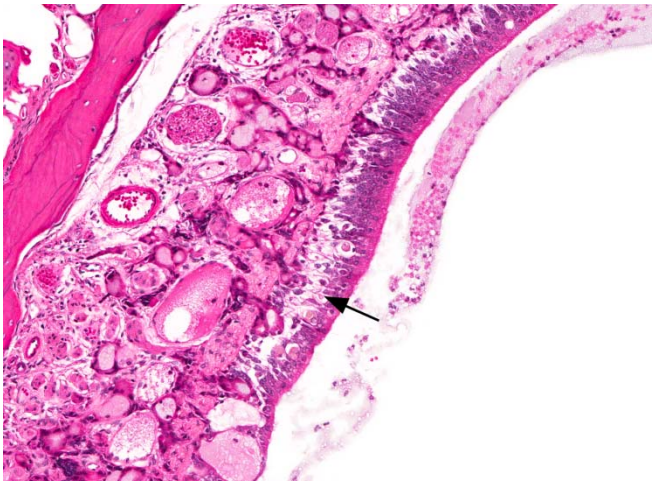


PLATE 19
Olfactory epithelium in a male F344/N rat administered 60 mg/kg *N*,*N*-dimethyl-*p*-toluidine by gavage for 2 years. Note the mild degeneration of the olfactory epithelium (arrow) as evidenced by decreased cellularity and vacuolization of the epithelial layer. There are few nerves present in the lamina propria. H&E

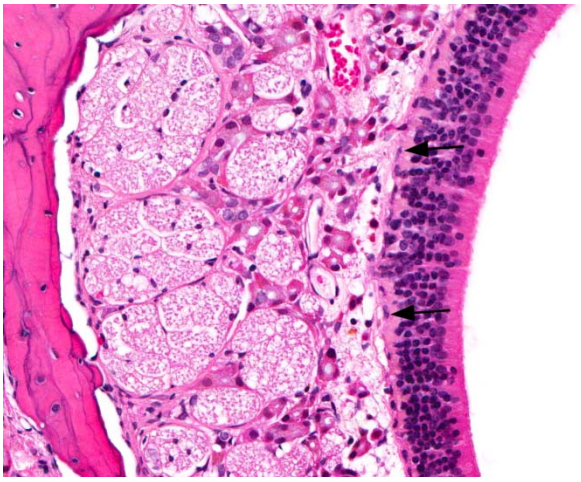


PLATE 20
Normal olfactory epithelium in a vehicle control male F344/N rat in the 2-year gavage study of *N*,*N*-dimethyl-*p*-toluidine. Note a row of flattened cells (arrows) at the basal layer of the epithelium. H&E

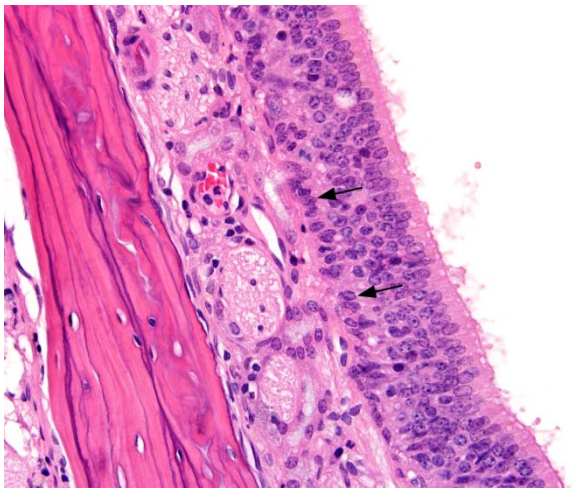


PLATE 21
Basal cell hyperplasia of the olfactory epithelium in a male F344/N rat administered 60 mg/kg *N*,*N*-dimethyl-*p*-toluidine by gavage for 2 years. Note the increased number of cells in the basal layer containing enlarged nuclei that are oriented perpendicular to the basal lamina (arrows). H&E

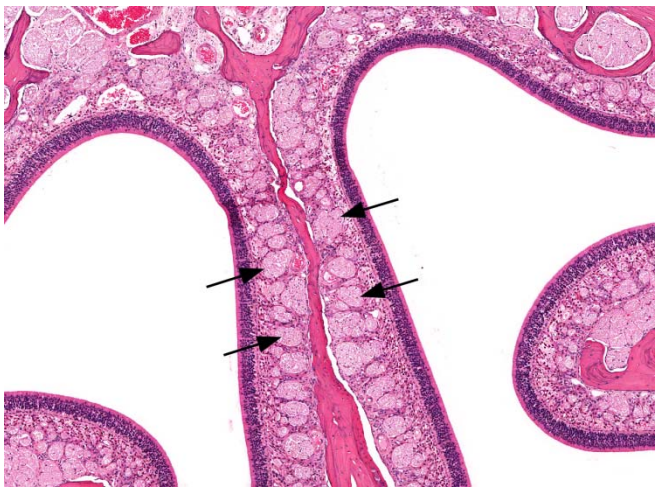


PLATE 22
Level III nasal section in a vehicle control male F344/N rat in the 2-year gavage study of *N*,*N*-dimethyl-*p*-toluidine showing the dorsal meatus and the dorsal septum lined by olfactory epithelium along with the underlying nerves (arrows). H&E

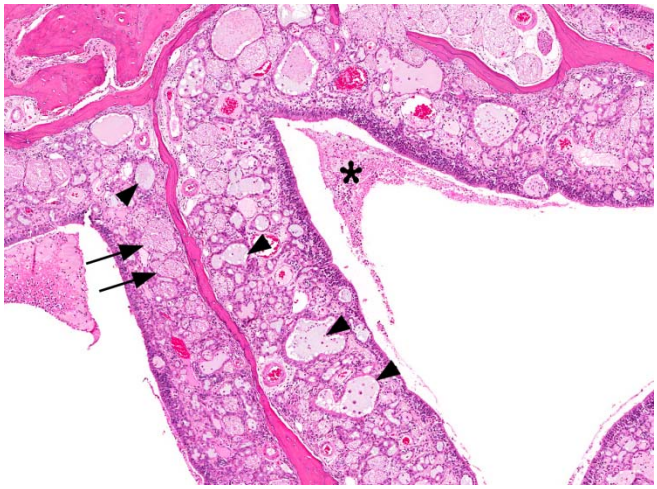


PLATE 23
Level III nasal section in a male F344/N rat administered 60 mg/kg *N*,*N*-dimethyl-*p*-toluidine by gavage for 2 years. Note the degeneration of the olfactory epithelium, dilatation of Bowman's glands (arrowheads), atrophy of the nerves (arrows), and inflammation (asterisk). H&E

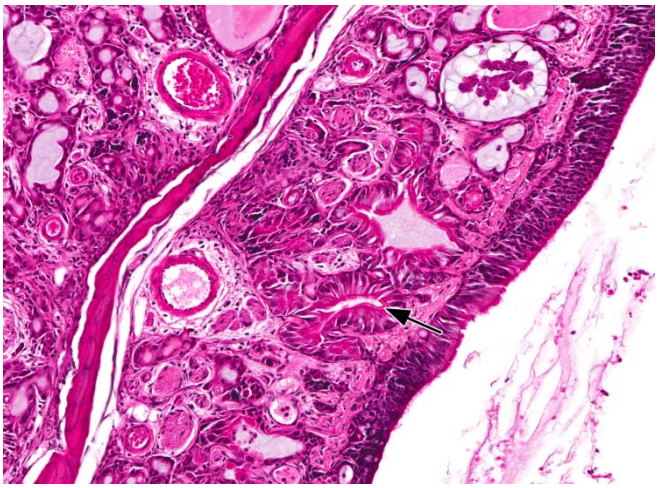


PLATE 24
Olfactory epithelium with hyperplasia and metaplasia (arrow) of the glands underlying the olfactory epithelium in a male F344/N rat administered 60 mg/kg *N*,*N*-dimthyl-*p*-toluidine by gavage for 2 years. H&E

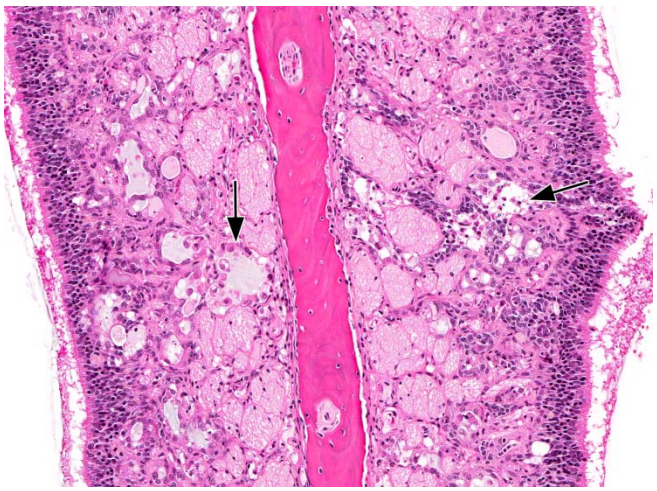


PLATE 25
Olfactory epithelium with necrotic glands (arrows) with individual necrotic cells present in the lumens of dilated glands in a male F344/N rat administered 60 mg/kg *N*,*N*-dimethyl-*p*-toluidine by gavage for 2 years. H&E

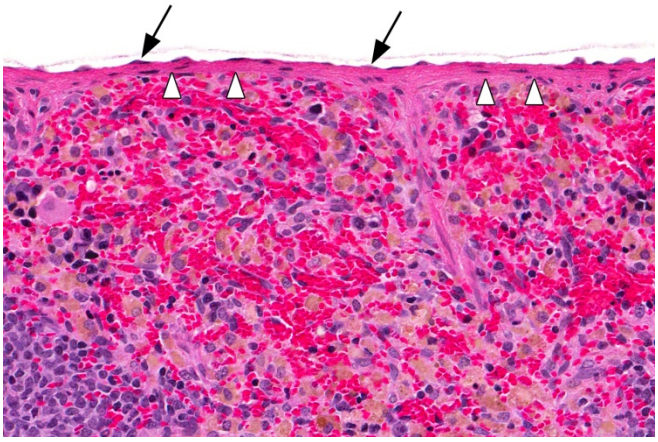


PLATE 26
Section of the spleen with a normal capsule (arrowheads) and mesothelium (arrows) in a vehicle control male F344/N rat in the 2-year gavage study of *N*,*N*-dimethyl-*p*-toluidine. H&E

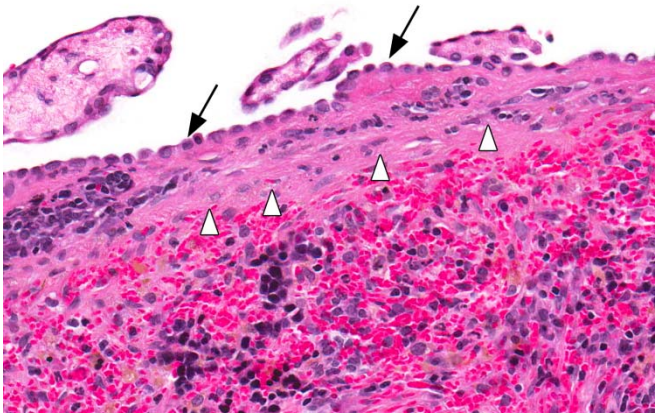


PLATE 27
Section of the spleen in a male F344/N rat administered 60 mg/kg *N*,*N*-dimethyl-*p*-toluidine by gavage for 2 years. Note the fibrosis of the splenic capsule (arrowheads) and hypertrophy of the mesothelium (arrows). H&E

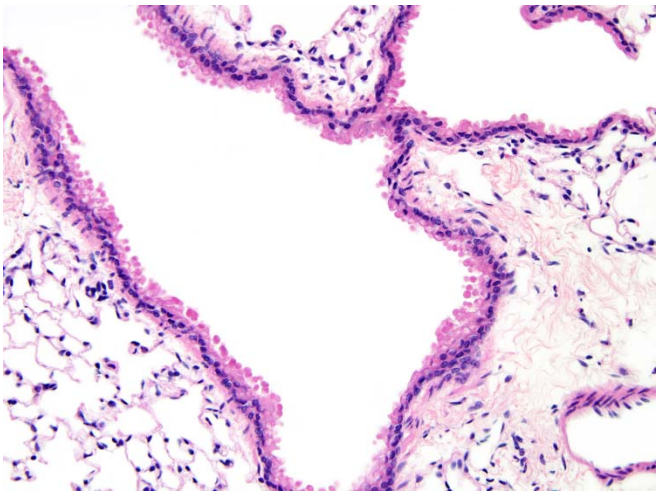


PLATE 28
Lung section from a vehicle control male B6C3F1/N mouse in the 3-month gavage study of *N*,*N*-dimethyl-*p*-toluidine. H&E

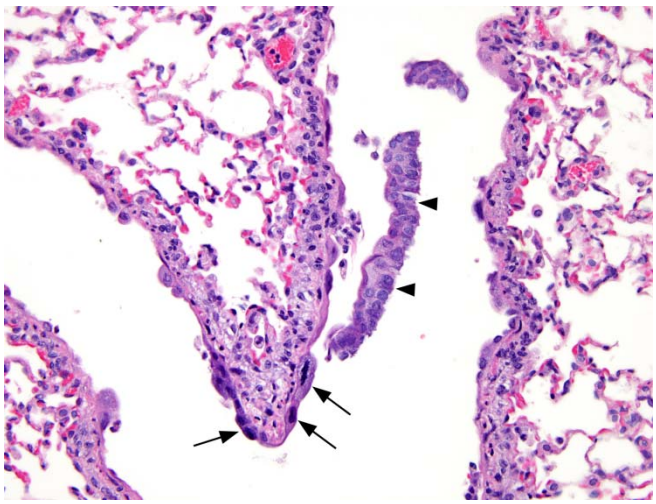


PLATE 29
Lung section from an early death male B6C3F1/N mouse administered 125 mg/kg *N*,*N*-dimethyl-*p*-toluidine in the 3-month gavage study. Note the sloughed epithelium (arrowheads) and regeneration of the bronchiolar epithelium (arrows). H&E

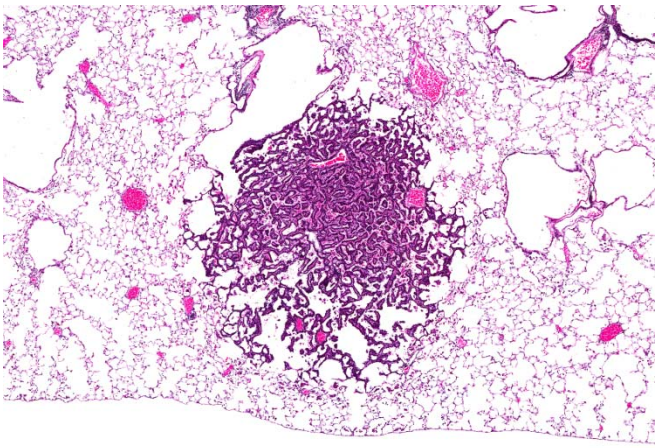


PLATE 30
An alveolar/bronchiolar adenoma in the lung of a female B6C3F1/N mouse administered 60 mg/kg *N*,*N*-dimethyl-*p*-toluidine by gavage for 2 years. The neoplasm is a discrete lesion causing compression of surrounding lung parenchyma, and composed of papillary projections lined by uniform populations of epithelial cells. H&E

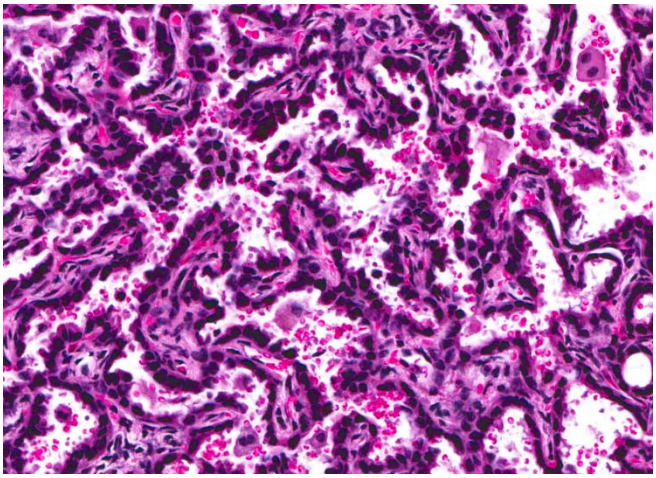


PLATE 31
Higher magnification of the alveolar/bronchiolar adenoma in Plate 28. H&E

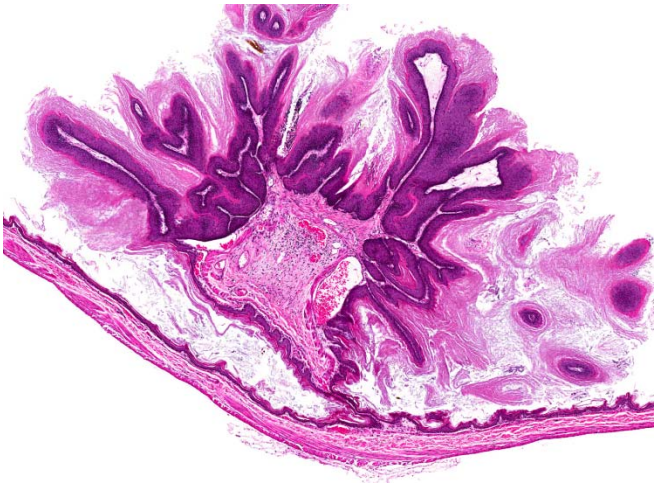


PLATE 32
A squamous cell papilloma of the forestomach in a female B6C3F1/N mouse administered 60 mg/kg *N*,*N*-dimethyl-*p*-toluidine by gavage for 2 years. H&E

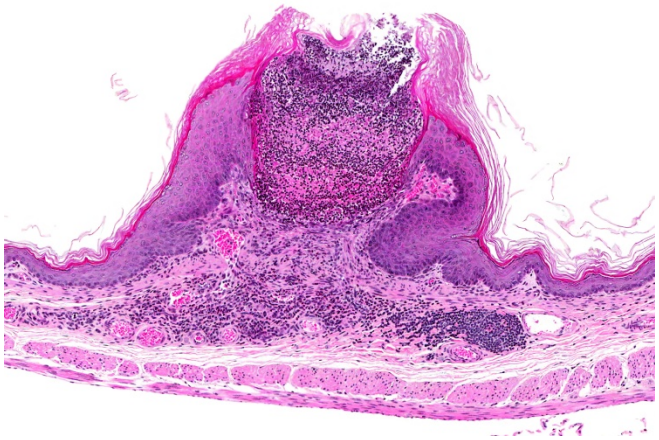


PLATE 33
Ulceration, inflammation, and epithelial hyperplasia of the forestomach in a female B6C3F1/N mouse administered 60 mg/kg *N*,*N*-dimethyl-*p*-toluidine by gavage for 2 years. H&E

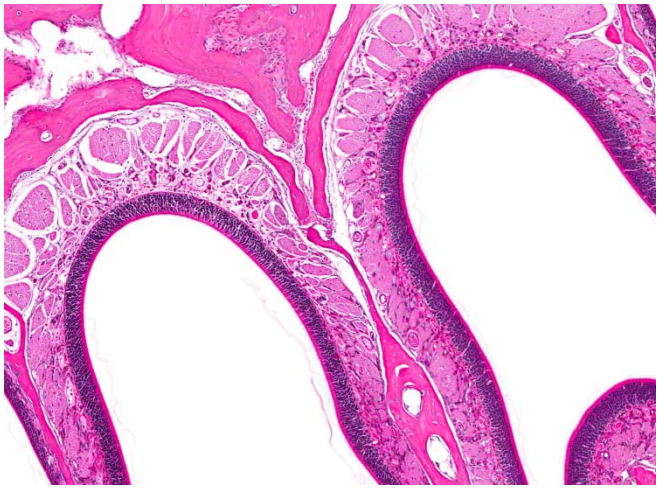


PLATE 34
Level III section of the nose showing the dorsal meatus and the dorsal septum lined by olfactory epithelium in a vehicle control B6C3F1/N mouse in the 2-year gavage study of *N*,*N*-dimethyl-*p*-toluidine. H&E

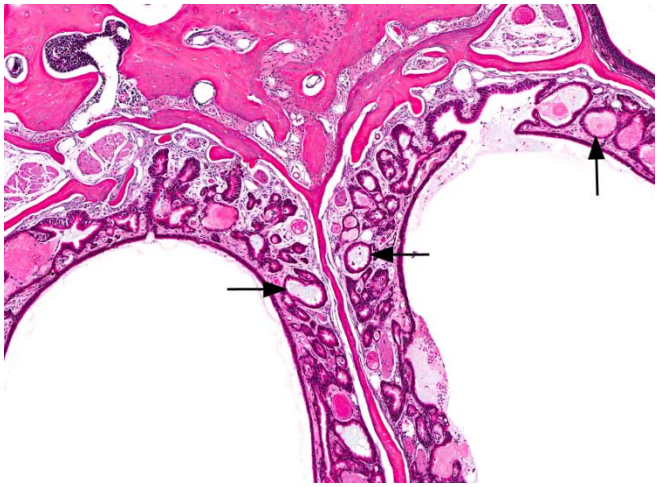


PLATE 35
Level III section of the nose in a female B6C3F1/N mouse administered 60 mg/kg *N*,*N*-dimethyl-*p*-toluidine by gavage for 2 years. Note the dilatation (arrows) and hyperplasia of the glands underlying the olfactory epithelium. H&E

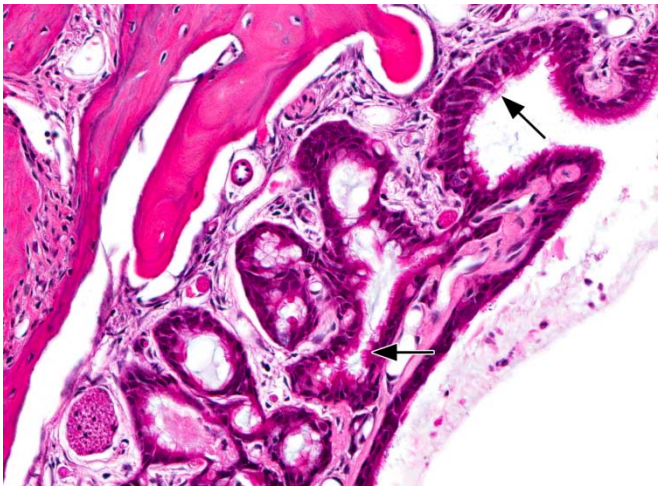


PLATE 36
Higher magnification of the Level III section of the nose in Plate 33. Note the respiratory metaplasia of the glands (arrows). H&E

DISCUSSION AND CONCLUSIONS

N,*N*-Dimethyl-*p*-toluidine is an accelerator in the redox initiator-accelerator system used commercially to cure methyl methacrylate monomers; polymerization is rarely complete. *N*,*N*-Dimethyl-*p*-toluidine is on the United States Environmental Protection Agency High Production Volume Chemical List with annual production estimates of greater than 1 million to less than 10 million pounds (USEPA, 2011a). Because of the potential for exposure to *N*,*N*-dimethyl-*p*-toluidine and the lack of toxicity and carcinogenicity information available in the literature, the National Toxicology Program (NTP) conducted toxicity and carcinogenicity studies in male and female F344/N rats and B6C3F1/N mice to help fill this data gap. In these studies, a treatment-related macrocytic regenerative anemia developed after 3 months of dosing in rats and mice. Nasal cavity, splenic, and liver toxicities were present in rats and mice after 3 months and 2 years of administration. Evidence for *N*,*N*-dimethyl-*p*-toluidine carcinogenic effects was seen in the liver of male and female rats and mice, the nasal cavity of male and female rats, and the lung and forestomach of female mice. *N*,*N*-Dimethyl-*p*-toluidine oxidative damage may be one mechanism contributing to these toxic and carcinogenic effects. *N*,*N*-Dimethyl-*p*-toluidine-induced methemoglobinemia is caused by oxidation of the heme moiety and this finding may be a sentinel response for other target organ toxicities caused by oxidative damage (Pallais *et al*., 2011). It has been demonstrated that *N*,*N*-dimethyl-*p*-toluidine is distributed to many of the sites where toxicity was observed in the current studies (Dix *et al.*, 2007; Kim *et al.*, 2007).

In the 3-month and 2-year studies, liver, nasal cavity, and hematologic toxicity was seen in rats and mice. In the 3-month rat and mouse studies, this toxicity included treatment-related decreases in body weights and increases in liver weights. Treatment-related lesions in the 3-month studies were seen in the liver, nose, kidney, hematopoietic system, and forestomach in rats and in the liver, lung, nose, and thymus in mice. The liver, kidney, and nasal tissues are target organs for acetaminophen-induced toxicity in rodents and the quinone imine intermediate is thought to be responsible for toxicity in these tissues (Genter *et al*., 1998). It could be speculated that *N*,*N*-dimethyl-*p*-toluidine formed a similar reactive metabolite that contributed to toxicity observed in these tissues in the current studies. In the rat study, treatment-related increases in alanine aminotransferase and sorbitol dehydrogenase activities and bile acid concentrations indicate a hepatic effect and were consistent with the histopathologic liver alterations and increased liver weights. In general, increases in serum activities of alanine aminotransferase and sorbitol dehydrogenase, considered liver-specific enzymes in rodents, are used as markers of hepatocellular necrosis or increased cell membrane permeability (Clampitt and Hart, 1978; Boyd, 1983). Increases of bile acid concentration are used as a marker of cholestasis (Hofmann, 1988; Hoffmann *et al*., 1989); however, they can also be affected by mechanisms other than cholestasis. For example, altered enterohepatic circulation, impaired hepatocellular function, and hepatocellular injury can increase circulating bile acid concentrations (Hofmann, 1988).

The nasal epithelial responses seen after oral gavage administration of *N*,*N*-dimethyl-*p*-toluidine in both rats and mice have been observed with other orally administered nitroaromatic compounds (NTP, 2002). One possible cause of the respiratory epithelial degeneration/necrosis is cytotoxicity as a result of pulmonary/nasal epithelial cytochrome P450 (CYP) metabolic activation of *N*,*N*-dimethyl-*p*-toluidine. The metabolic activation of acetaminophen by CYPs has been linked to olfactory epithelial damage in mice (Genter *et al*., 1998) and as previously stated, a similar reactive metabolite may be responsible for nasal toxicity in the current studies. Plopper *et al.* (1992) reported exfoliation and necrosis of the nonciliated cells in the terminal bronchioles and swelling/vacuolation of nonciliated cells of the lobar bronchus and trachea in mice administered naphthalene in corn oil by gavage. The NTP toxicity study of butanal oxime in B6C3F1 mice also described chemical-related nasal olfactory epithelium degeneration in the 3-month gavage study (NTP, 2004).

Toxicity to the reproductive system was demonstrated by an extended diestrus in female rats administered 125 or 250 mg/kg when compared to vehicle controls. Decreased epididymis and testis weights were seen in male rats administered 250 mg/kg. These changes show the potential for reproductive toxicity and are probably not related to decreases in body weight or systemic

toxicity (Chapin *et al*., 1993). There were no significant effects on reproductive parameters measured in mice.

In the 3-month rat study, a macrocytic, hypochromic, responsive Heinz body anemia increased in severity with increasing dose of *N*,*N*-dimethyl-*p*-toluidine. This anemia was characterized by decreased hematocrit values, hemoglobin concentrations, and erythrocyte counts, and increased mean cell volumes, methemoglobin values, and Heinz body production in all dosed groups. The mechanism for the anemia is thought to involve oxidative damage to erythrocytes leading to methemoglobin and Heinz body formation and resulting in decreased erythrocyte survival. This effect is similar to methemoglobin-induced anemias in animals and humans associated with exposure to other aniline and nitroaromatic compounds (Finch, 1948; Smith, 1996). In NTP studies of nitroaromatic chemicals, regenerative anemia and methemoglobinemia also occurred in rats (Dunnick *et al*., 1994; Travlos *et al*., 1996). Methemoglobinemia was also seen in Wistar rats administered *p*-toluidine by oral gavage at doses of 40, 80, or 160 mg/kg for 13 weeks (Jodynis-Liebert and Bennasir, 2005). Splenic changes observed in rats (congestion and pigmentation) have been attributed to the denaturation of hemoglobin, Heinz body formation, and increased erythrocyte destruction in the spleen (NTP, 1996a).

In mice, administration of *N*,*N*-dimethyl-*p*-toluidine at equivalent doses caused similar, but much less severe, erythron changes compared to rats. Methemoglobin and Heinz body formation occurred, but consistent decreases in the circulating erythroid mass were not evident. In fact, for female mice, no erythron changes were detected up to the 125 mg/kg dose. The amount of methemoglobin formed in dosed mice was lower than that observed in rats. The lower methemoglobin levels in mice may be due in part to a higher methemoglobin reductase activity than in rats (Stolk and Smith, 1966; Car *et al.*, 2006) or may occur as the result of quantitative differences in *N*,*N*-dimethyl-*p*-toluidine metabolism between the two species.

Analysis of hematologic endpoints in clinical pathology groups of male and female rats at 3 months into the 2-year study also showed the development of a macrocytic regenerative anemia as measured by increases in mean cell volume and Heinz body formation and decreases in erythrocyte counts and hematocrit and hemoglobin serum levels at 20 and 60 mg/kg; hemoglobin in 6 mg/kg female rats was also significantly decreased. Increases in methemoglobin serum levels occurred in all dosed male rat groups and in 20 and 60 mg/kg female rats. Methemoglobinemia has been reported in a human after an accidental ingestion of *N*,*N*-dimethyl-*p*-toluidine at an estimated level of 6 mg/kg body weight (Potter *et al*., 1988). While hematologic toxicity was observed in rats, there were no increases in the incidences of hematologic tumors.

The hematology results of these 3-month gavage studies (including the 3-month interim evaluation in the 2-year study) indicated that exposure of rats and mice (to a lesser extent) to *N*,*N*-dimethyl-*p*-toluidine affected the circulating erythroid mass. Methemoglobinemia appeared to be the primary toxic response, and many of the other lesions described in the current studies could be explained as secondary to methemoglobin formation and subsequent increases in oxidative erythrocyte injury and turnover. Lesions included responsive anemia, red cell morphologic alterations (Heinz bodies), and increased hematopoietic cell proliferation in the spleen and bone marrow.

Doses for the 2-year *N*,*N*-dimethyl-*p*-toluidine studies were selected based on the findings from the 3-month studies. Because of the decreases in mean body weights in rats (greater than 10% decrease compared to the vehicle controls) and target organ toxicity in rats and mice (including liver, spleen, and nasal cavity toxicities and hematologic toxicity) at doses greater than 125 mg/kg in the 3-month studies, the high dose selected for the 2-year rat and mouse studies was 60 mg/kg. The 2-year dose range (6 to 60 mg/kg) overlapped an accidental *N*,*N*-dimethyl-*p*-toluidine exposure in a child (Potter *et al.*, 1988).

In the 2-year studies, decreases in mean body weight were seen in 60 mg/kg male and female rats and mice compared to vehicle controls. The survival of 60 mg/kg male rats was significantly less than that of the vehicle controls; the cause of some of the early deaths in this group was related to the development of treatment-related neoplasms in the nose or liver.

Toxicity in the nasal cavity and liver toxicity occurred in male and female rats and mice in the 2-year studies. The liver histopathologic findings included hepatocellular hypertrophy in 20 and 60 mg/kg rats and in all dosed groups of mice. Hyperplasia of the olfactory, respiratory, and transitional epithelia of the nose occurred in rats while in mice, hyperplasia and/or metaplasia were seen only in the olfactory and respiratory epithelia. Splenic lesions including congestion, hematopoietic cell proliferation, hypertrophy, and fibrosis were prominent in dosed rats, but did not occur in mice. In addition, toxicity was seen in the kidney [increase in severity of nephropathy (male and female rats)], forestomach [hyperplasia and ulcer (male rats and female mice)], and lung (female mice).

Beginning at month 8 in the 2-year study, rats began to exhibit hyperactivity and boxing behavior, often described as a defensive behavior (Bataineh and Nusier, 2006). Other studies have shown that nasal toxicity may alter behavioral responses (Gelhaye *et al.*, 2006). Treatment-related clinical signs were not reported in dosed mice in the 2-year study.

The treatment-related increases in liver neoplasms in male and female rats and mice were considered to be clear evidence for a carcinogenic response because of the significant increases in malignant neoplasms that exceeded the incidences in the current vehicle controls and the ranges of historical controls. In male and female rats, this included increases in hepatocellular carcinoma and hepatocellular adenoma or carcinoma (combined). In mice, this included increases in hepatocellular adenoma (increases in multiple hepatocellular adenoma in males and increases in multiple and total incidences of hepatocellular adenoma in females); increases in multiple and the total incidences of hepatocellular carcinoma in males and females; increases in hepatoblastoma in males and females; and increases in hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) in males and females.

Hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma represent a biological and morphological continuum (Takahashi *et al.*, 2002); therefore, it is appropriate to combine the incidences of hepatoblastoma with those of hepatocellular adenoma and hepatocellular carcinoma when interpreting the carcinogenic potential of a chemical. Hepatoblastomas are rare spontaneous neoplasms that may occur after chemical administration (primarily in mice) and have previously been seen in NTP studies of benzofuran, ethylene thiourea, *o*-nitroanisole, coumarin, methylphenidate hydrochloride, 1-amino-2,4-dibromoanthraquinone, oxazepam, pyridine, primidone, and goldenseal (NTP, 1989, 1992, 1993a,b, 1995, 1996b, 1998, 2000a,b, 2010). They often arise from hepatocellular neoplasms and when this occurs, only the hepatoblastoma is diagnosed. Hepatoblastomas in humans account for approximately 70% of childhood liver cancers (Darbari *et al.*, 2003).

In the nose of male rats there were increased incidences of nasal cavity neoplasms, primarily transitional epithelium neoplasms accompanied by increased incidences of nonneoplastic nasal cavity lesions including hyperplasia of the olfactory, respiratory, and transitional epithelia. In male rats, the incidence of transitional epithelium adenoma was significantly increased in the 60 mg/kg group. Two transitional epithelium carcinomas occurred in the 60 mg/kg group. A few transitional epithelium adenomas also occurred in the 6 and 20 mg/kg groups, and the trend of incidences of this nasal cavity neoplastic response was statistically significant. Transitional epithelium neoplasms of the nasal cavity have not been seen in 1,248 historical control male rats from all routes of exposure. The occurrence of these rare nasal cavity neoplasms was considered to be clear evidence of a carcinogenic effect.

The occurrence of nasal cavity transitional epithelium adenoma in female rats was considered to be related to treatment because these are rare neoplasms that have not occurred in the concurrent vehicle controls or in corn oil gavage historical controls and have occurred in only one of 1,196 historical control animals by all routes of exposure. In addition, the evidence for a *N,N*-dimethyl-*p*-toluidine-induced nasal neoplasm response in female rats was supported by the finding of a treatment-related neoplastic response in the nasal cavity of male rats.

The occurrence of thyroid gland follicular cell neoplasms in male rats may have been related to treatment. The incidence of thyroid gland follicular cell adenoma was increased at the 60 mg/kg dose, and in addition, two thyroid gland follicular cell carcinomas occurred in the 60 mg/kg group. The incidence of follicular cell adenoma or carcinoma (combined) in the 60 mg/kg group exceeded the historical control ranges for corn oil gavage studies and for all routes of exposure.

In the forestomach of female mice, there were significantly increased incidences of squamous cell papilloma and squamous cell papilloma or carcinoma (combined). The incidences of these neoplasms in the 20 and 60 mg/kg groups exceeded the historical control ranges for corn oil gavage controls and for all routes of exposure. This was considered to be some evidence for a carcinogenic effect rather than clear evidence because it was noted that the incidence of these neoplasms in vehicle controls was lower than the mean incidence for the corn oil gavage historical controls and these neoplasms were primarily not malignant neoplasms.

The incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined) in the lung of female mice were significantly increased in the 20 and 60 mg/kg groups. This neoplasm response was considered to be clear evidence for a carcinogenic response. The incidence of alveolar/bronchiolar adenoma or carcinoma (combined) in the 60 mg/kg group exceeded the historical control ranges for corn oil gavage studies and for all routes of exposure. This finding was supported by the occurrence of nonneoplastic lung lesions.

N,N-Dimethyl-*p*-toluidine and other nitroaromatic chemicals (NTP, 2002) cause hematopoiesis and hemosiderin pigment accumulation in the spleen and decreased incidences of mononuclear cell leukemia in male rats (Elwell *et al.*, 1996). The spleen plays a critical role in the pathogenesis of mononuclear cell leukemia. Although the stem cell for mononuclear cell leukemia in Fischer rats is considered to be a lymphocyte of bone marrow origin, the initial histological evidence for proliferation and expansion of these neoplastic cells occurs in the spleen, where the leukemia cells fill the sinusoids. Alteration of the spleen microenvironment can affect the development of mononuclear cell leukemia (Elwell *et al.*, 1996). The findings of increased splenic toxicity and decreased incidences of mononuclear cell leukemia in male and female rats in the current study are consistent with similar response patterns seen with other nitroaromatic compounds, including *p*-nitrotoluene (Elwell *et al.*, 1996).

When the National Cancer Institute (NCI) nominated *N,N*-dimethyl-*p*-toluidine for study they noted that the NTP/NCI had conducted cancer bioassays with structurally related chemicals and these chemicals were carcinogenic in rats and/or mice, particularly *ortho*-substituted aromatic compounds. *o*-Toluidine hydrochloride induced a high incidence of mesothelioma in male rats, while no mesotheliomas were observed after treatment with *m*- or *p*-toluidine (Weisburger *et al.*, 1978). In studies of *o*- and *p*-anisidine hydrochloride, only the *ortho* isomer induced a carcinogenic response (urinary bladder neoplasms in rats and mice and kidney and thyroid gland neoplasms in male rats) (NCI, 1978a,b). *o*-Toluidine hydrochloride, *o*-anisidine hydrochloride, and *o*-nitrotoluene [also inducing a wide range of carcinogenic effects including mesotheliomas in rats (NTP, 2002)] all contain a benzene ring with a methyl- or methoxy- and nitrogen-containing group on adjacent carbons. This structural similarity suggests that a similar intermediate may be responsible for the carcinogenic effects. *N,N*-Dimethyl-*p*-toluidine did not have the same spectrum of carcinogenic activity as these other aminoaromatic chemicals, suggesting different mechanisms may have been involved with the carcinogenic activity.

Like *N,N*-dimethyl-*p*-toluidine, *p*-toluidine induced liver neoplasms in mice fed diets containing the chemical at concentrations of 1,000 or 2,000 ppm for 6 months, and then diets containing the chemical at 500 or 1,000 ppm (respectively) for an additional 12 months (Weisberger *et al.*, 1978). Details on the types of liver neoplasms were not reported. *p*-Toluidine was not reported to induce neoplasms in Sprague-Dawley rats although the animals were only exposed to the chemical (1,000 or 2,000 ppm) for 18 months (Weisburger *et al*., 1978).

N,N-Dimethyl-*p*-toluidine was not mutagenic in any of several bacterial tester strains, with or without exogenous metabolic activation, but positive results were reported in an *in vitro* micronucleus assay conducted in Chinese hamster ovary cells (Taningher *et al*., 1993), and an *in vitro* gene mutation assay in L5178Y mouse lymphoma $tk^{+/-}$ cells (IARC, 1999). In both cases, positive results were seen in the absence of metabolic activation, suggesting that *N,N*-dimethyl-*p*-toluidine was a direct-acting mutagenic agent. In the current study, however, no increases in the frequencies of micronucleated reticulocytes or erythrocytes were seen in male and/or female mice following 4 days or 3 months of exposure to *N,N*-dimethyl-*p*-toluidine by gavage (Appendix E). Furthermore, no increases in DNA damage were observed in liver preparations from the male mice treated with *N,N*-dimethyl-*p*-toluidine for 4 days. However, a small but statistically significant (P=0.024) increase in DNA damage was observed by the comet assay in liver cells of male Sprague-Dawley rats administered 60 mg/kg *N,N*-dimethyl-*p*-toluidine by gavage once daily for 4 days. An analysis of results of a comprehensive study of chemical-induced DNA damage measured by the comet assay in mice and rats showed that a significant increase in DNA damage in at least one organ of one species is well correlated with rodent carcinogenicity (Sasaki *et al*., 2000). The authors further concluded that the species or tissue in which chemical-induced DNA damage was detected did not always predict the species or tissue in which tumors were seen.

The carcinogenic effects of *N,N*-dimethyl-*p*-toluidine in the nasal cavity (male rats), liver (male and female rats and mice), and lung and forestomach (female mice) may be due in part to toxicity and increased cell turnover in target organs. In a human submandibular gland adenocarcinoma cell line with visible light irradiation, the photosensitizer camphorquinone in the presence of *N,N*-dimethyl-*p*-toluidine demonstrated both dose- and time-dependent production of reactive oxygen species (Atsumi *et al*., 2001). Free radical formation with subsequent DNA damage in target organs may also have contributed to the carcinogenic effects (Winter *et al.*, 2005; Masuki *et al.*, 2007; Li *et al.*, 2008; Pereira *et al.*, 2008). *N,N*-Dimethyl-*p*-toluidine has been reported to have an estrogen antagonist activity *in vitro* (yeast reporter gene assay) (Nomura *et al.*, 2003). Whether this reported endocrine effect contributed to *N,N*-dimethyl-*p*-toluidine biologic effects is not known. Members of the alkylaniline class of compounds, including *p*-toluidine (4-methylaniline), have the potential for DNA adduct formation (Marques *et al*., 1997), although the DNA binding capacity of *N,N*-dimethyl-*p*-toluidine was not measured in this series of studies.

CONCLUSIONS

Under the conditions of these 2-year oral gavage studies, there was *clear evidence of carcinogenic activity** of *N,N*-dimethyl-*p*-toluidine in male F344/N rats based on increased incidences of hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined), and increased incidences of nasal cavity neoplasms (primarily nasal cavity transitional epithelium adenoma). The increased incidences of thyroid gland follicular cell neoplasms may have been related to treatment. There was *clear evidence of carcinogenic activity* of *N,N*-dimethyl-*p*-toluidine in female F344/N rats based on increased incidences of hepatocellular carcinoma and hepatocellular adenoma or carcinoma (combined). The occurrence of nasal cavity transitional epithelium adenoma was considered to be related to treatment. There was *clear evidence of carcinogenic activity* of *N,N*-dimethyl-*p*-toluidine in male B6C3F1/N mice based on increased incidences of hepatocellular adenoma (multiple), hepatocellular carcinoma, and hepatoblastoma. There was *clear evidence of carcinogenic activity* of *N,N*-dimethyl-*p*-toluidine in female B6C3F1/N mice based on increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma and increased incidences of alveolar/bronchiolar neoplasms (primarily adenoma). The increased incidences of forestomach squamous cell papilloma in female mice were considered to be related to treatment.

Administration of *N,N*-dimethyl-*p*-toluidine resulted in increased incidences of nonneoplastic lesions of the liver and nasal cavity in male and female rats and mice; the kidney in male and female rats; the spleen and bone marrow in male and female rats and female mice; the lung in male and female mice; the forestomach in male rats and female mice; the mesenteric lymph node in male rats and female mice; and the olfactory lobe in male and female mice.

N,N-Dimethyl-*p*-toluidine also caused hematologic toxicity and increases in methemoglobin levels in male and female rats and mice (as measured at 3 months).

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 14. A summary of the Peer Review Panel comments and the public discussion on this Technical Report appears on page 16.

REFERENCES

3M (2004). 3M™ Scotch-Weld™ Instant Adhesive Surface Activator: Material Safety Data Sheet. 3M, St. Paul, MN.

The Aldrich Library of FT-IR Spectra (1997). 2nd ed. (C.J. Pouchert, Ed.). Spectrum II:2042D. Aldrich Chemical Company, Inc., Milwaukee, WI.

Ashby, J., and Tennant, R.W. (1991). Definitive relationships among chemical structure, carcinogenicity and mutagenicity for 301 chemicals tested by the U.S. NTP. *Mutat. Res.* **257**, 229-306.

Atsumi, T., Iwakura, I., Fujisawa, S., and Ueha, T. (2001). The production of reactive oxygen species by irradiated camphorquinone-related photosensitizers and their effect on cytotoxicity. *Arch. Oral Biol*. **46**, 391-401.

Bailer, A.J., and Portier, C.J. (1988). Effects of treatment-induced mortality and tumor-induced mortality on tests for carcinogenicity in small samples. *Biometrics* **44**, 417-431.

Bataineh, H.N., and Nusier, M.K. (2006). Impact of 12-week ingestion of sodium fluoride on aggression, sexual behavior, and fertility in adult male rats. *Fluoride* **39**, 293-301.

Bieler, G.S., and Williams, R.L. (1993). Ratio estimates, the delta method, and quantal response tests for increased carcinogenicity. *Biometrics* **49**, 793-801.

Boorman, G.A., Montgomery, C.A., Jr., Eustis, S.L., Wolfe, M.J., McConnell, E.E., and Hardisty, J.F. (1985). Quality assurance in pathology for rodent carcinogenicity studies. In *Handbook of Carcinogen Testing* (H.A. Milman and E.K. Weisburger, Eds.), pp. 345-357. Noyes Publications, Park Ridge, NJ.

Bösch, P., Harms, H., and Lintner, F. (1982). The proof of dimethylparatoluidine in bone cement even after long-time implantation [in German, English summary]. *Arch. Toxicol.* **51**, 157-166.

Boyd, J.W. (1983). The mechanisms relating to increases in plasma enzymes and isoenzymes in diseases of animals. *Vet. Clin. Pathol.* **12**, 9-24.

Brauer, G.M., Termini, D.J., and Dickson, G. (1977). Analysis of the ingredients and determination of the residual components of acrylic bone cements. *J. Biomed. Mater. Res*. **11**, 577-607.

Brauer, G.M., Steinberger, D.R., and Stansbury, J.W. (1986). Dependence of curing time, peak temperature, and mechanical properties on the composition of bone cement. *J. Biomed. Mater. Res.* **20**, 839-852.

Brecher, G., and Schneiderman, M. (1950). A time-saving device for the counting of reticulocytes. *Am. J. Clin. Pathol.* **20**, 1079-1083.

Burlinson, B., Tice, R.R., Speit, G., Agurell, E., Brendler-Schwaab, S.Y., Collins, A.R., Escobar, P., Honma, M., Kumaravel, T.S., Nakajima, M., Sasaki, Y.F., Thybaud, V., Uno, Y., Vasquez, M., and Hartmann, A. (2007). Fourth International Workingroup on Genotoxicity Testing: Results of the *in vivo* Comet assay workgroup. *Mutat. Res.* **627**, 31-35.

Car, B.D., Eng, V.M., Everds, N.E., and Bounous, D.I. (2006). Clinical pathology of the rat. In *The Laboratory Rat*, 2nd ed. (M.A. Suckow, S.H. Weisbrouth, and C.L. Franklin, Eds.), pp. 128-149. Elsevier, Inc., Burlington, MA.

Chapin, R.E., Gulati, D.K., Barnes, L.H., and Teague, J.L. (1993). The effects of feed restriction on reproductive function in Sprague-Dawley rats. *Fundam. Appl. Toxicol.* **20**, 23-29.

ChemFirst, Inc. (1997). Initial submission: Acute inhalation toxicity study of N,N-dimethyl-p-toluidine in rats with MSDS attachment. EPA/OTS: Doc-88970000118S.

Clampitt, R.B., and Hart, R.J., (1978). The tissue activities of some diagnostic enzymes in ten mammalian species. *J. Comp. Pathol*. **88**, 607-621.

Code of Federal Regulations (CFR) **21**, Part 58.

Collins, A.R., Oscoz, A.A., Brunborg, G., Gaivão, I., Giovannelli, L., Kruszewski, M., Smith, C.C., and Štětina, R. (2008). The comet assay: Topical issues. *Mutagenesis* **23**, 143-151.

Cox, D.R. (1972). Regression models and life-tables. *J. R. Stat. Soc.* **B34**, 187-220.

Crawford, B.D. (1985). Perspectives on the somatic mutation model of carcinogenesis. In *Advances in Modern Environmental Toxicology. Mechanisms and Toxicity of Chemical Carcinogens and Mutagens* (M.A. Mehlman, W.G. Flamm, and R.J. Lorentzen, Eds.), pp. 13-59. Princeton Scientific Publishing Co., Inc., Princeton, NJ.

Darbari, A., Sabin, K.M., Shapiro, C.N., and Schwarz, K.B. (2003). Epidemiology of primary hepatic malignancies in U.S. children. *Hepatology* **38**, 560-566.

Dix, K.J., Ghanbari, K., and Hedtke-Weber, B.M. (2007). Disposition of [^{14}C]*N,N*-dimethyl-*p*-toluidine in F344 rats and B6C3F1 mice. *J. Toxicol. Environ. Health A* **70**, 789-798.

Dixon, W.J., and Massey, F.J., Jr. (1957). *Introduction to Statistical Analysis*, 2nd ed., pp. 276-278, 412. McGraw-Hill Book Company, Inc., New York.

Druckrey, H., Schmähl, D., and Reiter, A. (1954). Fehlen einer carcerogenen Wirking bei den drei Isomeren N-dimethyl-toluidinen an Ratten. *Arzneimittelforschung* **4**, 365-366.

Dunn, O.J. (1964). Multiple comparisons using rank sums. *Technometrics* **6**, 241-252.

Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* **50**, 1096-1121.

Dunnick, J.K., Elwell, M.R., and Bucher, J.R. (1994). Comparative toxicities of *o*-, *m*-, and *p*-nitrotoluene in 13-week feed studies in F344 rats and B6C3F$_1$ mice. *Fundam. Appl. Toxicol.* **22**, 411-421.

Dutrée-Meulenberg, R.O.G.M., Kozel, M.M.A., and van Joost, Th. (1992). Burning mouth syndrome: A possible etiologic role for local contact hypersensitivity. *J. Am. Acad. Dermatol.* **26**, 935-940.

Elwell, M.R., Dunnick, J.K., Hailey, J.R., and Haseman, J.K. (1996). Chemicals associated with decreases in the incidence of mononuclear cell leukemia in the Fischer rat. *Toxicol. Pathol.* **24**, 238-245.

Evelyn, K.A., and Malloy, H.T. (1938). Microdetermination of oxyhemoglobin, methemoglobin and sulfhemoglobin in a single sample of blood. *J. Biol. Chem.* **126**, 655-662.

Finch, C.A. (1948). Methemoglobinemia and sulfhemoglobinemia. *N. Engl. J. Med*. **239**, 470-478.

Fomby, L., and Graves, S. (2001). Regarding NTP ChemTask No. CHEM06191, Battelle Study No. G004110-AYJ, CAS No., 99-97-8, Preliminary Chemical Studies Task: Palatability study for N,N-dimethyl-*p*-toluidine (DMPT) formulated in feed and administered to Fischer 344 rats and B6C3F1 mice for up to 14 days. Letter dated June 18, 2001, to Dr. Cynthia Smith, NIEHS. Battelle Memorial Institute, Columbus, OH.

Gart, J.J., Chu, K.C., and Tarone, R.E. (1979). Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. *JNCI* **62**, 957-974.

Geiger, D.L., Poirier, S.H., Brooke, L.T., and Call, D.J., Eds. (1986). *Acute Toxicities of Organic Chemicals to Fathead Minnows* (*Pimephales promelas*), Volume III, pp. 221-224. Center for Lake Superior Environmental Studies, University of Wisconsin-Superior, Superior, WI.

Gelhaye, M., Trabalon, M., Martrette, J.M., and Legrand-Frossi, C. (2006). Effects of early olfactory deprivation on novelty-seeking behavior and primary and secondary lymphoid organs in young rats. *Psychoneuroendocrinology* **31**, 997-1008.

Genter, M.B., Liang, H.C., Gu, J., Ding, X., Negishi, M., McKinnon, R.A., and Nebert, D.W. (1998). Role of CYP2A5 and 2G1 in acetaminophen metabolism and toxicity in the olfactory mucosa of the Cyp1a2(-/-) mouse. *Biochem. Pharmacol.* **55**, 1819-1826.

Ghanayem, B.I., Witt, K.L., Kissling, G.E., Tice, R.R., and Recio, L. (2005). Absence of acrylamide-induced genotoxicity in CYP2E1-null mice: Evidence consistent with a glycidamide-mediated effect. *Mutat. Res.* **578**, 284-297.

Girard, D.M., and Sager, D.B. (1987). The use of Markov chains to detect subtle variation in reproductive cycling. *Biometrics* **43**, 225-234.

Gorrod, J.W., and Gooderham, N.J. (1981). The *in vitro* metabolism of N,N-dimethylaniline by guinea pig and rabbit tissue preparations. *Eur. J. Drug Metab. Pharmacokinet.* **6**, 195-206.

Haddad, F.S., Levell, N.J., Dowd, P.M., Cobb, A.G., and Bentley, G. (1995). Cement hypersensitivity: A cause of aseptic loosening? *J. Bone Joint Surg. [Br]* **77**, 329-330.

Haddad, F.S., Cobb, A.G., Bentley, G., Levell, N.J., and Dowd, P.M. (1996). Hypersensitivity in aseptic loosening of total hip replacements. *J. Bone Joint Surg. [Br]* **78**, 546-549.

Harrison, J.H., Jr., and Jollow, D.J. (1987). Contribution of aniline metabolites to aniline-induced methemoglobinemia. *Mol. Pharmacol.* **32**, 423-431.

Haseman, J.K., Young, E., Eustis, S.L., and Hailey, J.R. (1997). Body weight-tumor incidence correlations in long-term rodent carcinogenicity studies. *Toxicol. Pathol.* **25**, 256-263.

Heddle, J.A., Hite, M., Kirkhart, B., Mavournin, K., MacGregor, J.T., Newell, G.W., and Salamone, M.F. (1983). The induction of micronuclei as a measure of genotoxicity. A report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res.* **123**, 61-118.

Hirabayashi, C. (2003). Studies on MMA-TBB resin II. The effect of dual use of TBB and other initiators on polymerization of PMMA/MMA resin. *Dent. Mater. J.* **22**, 48-55.

Hirabayashi, C., and Imai, Y. (2002). Studies on MMA-TBB resin I. Comparison of TBB and other initiators in the polymerization of PMMA/MMA resin. *Dent. Mater. J.* **21**, 314-321.

Hoffmann, W.E., Kramer, J., Main, A.R., and Torres, J.L. (1989). Clinical enzymology. In *The Clinical Chemistry of Laboratory Animals* (W.F. Loeb and F.W. Quimby, Eds.), pp. 237-278. Pergamon Press, Inc., New York.

Hofmann, A.F. (1988). Bile acids. In *The Liver: Biology and Pathobiology*, 2nd ed. (I.M. Arias, W.B. Jakoby, H. Popper, D. Schachter, and D.A. Shafritz, Eds.), pp. 553-572. Raven Press, Ltd., New York.

International Agency for Research on Cancer (IARC) (1999). *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Surgical Implants and Other Foreign Bodies*, Vol. 74. IARC, Lyon, France.

Jodynis-Liebert, J., and Bennasir, H.A.H. (2005). Effect of dietary fat on selected parameters of toxicity following 1- or 3-month exposure of rats to toluidine isomers. *Int. J. Toxicol.* **24**, 365-376.

Jonckheere, A.R. (1954). A distribution-free *k*-sample test against ordered alternatives. *Biometrika* **41**, 133-145.

Kaaber, S. (1990). Allergy to dental materials with special reference to the use of amalgam and polymethylmethacrylate. *Int. Dent. J.* **40**, 359-365.

Kaaber, S., Thulin, H., and Nielsen, E. (1979). Skin sensitivity to denture base materials in the burning mouth syndrome. *Contact Dermatitis* **5**, 90-96.

Kao, L., Leikin, J.B., Crockett, M., and Burda, A. (1997). Methemoglobinemia from artificial fingernail solution. *JAMA* **278**, 549-550.

Kaplan, E.L., and Meier, P. (1958). Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc.* **53**, 457-481.

Kendall, M.G. (1938). A new measure of rank correlation. *Biometrika* **30**, 81-93.

Kim, N.-C., Ghanbari, K., Kracko, D.A., Weber, W.M., McDonald, J.D., and Dix, K.J. (2007). Identification of urinary metabolites of orally administered *N,N*-dimethyl-*p*-toluidine in male F344 rats. *J. Toxicol. Environ. Health A* **70**, 781-788.

Kissling, G.E., Dertinger, S., Hayashi, M., and MacGregor, J.T. (2007). Sensitivity of the erythrocyte micronucleus assay: Dependence on number of cells scored and inter-animal variability. *Mutat. Res.* **634**, 235-240.

Kronoveter, K.J. (1977). Health Hazard Evaluation Determination Report No. HHE-76-82-361. Hair Zoo, Penfield, NY. Division of Surveillance, Hazard Evaluation, and Field Studies, National Institute of Occupational Safety and Health, Cincinnati, OH.

Lewis, G. (2008). Alternative acrylic bone cement formulations for cemented arthroplasties: Present status, key issues, and future prospects. *J. Biomed. Mater. Res. B Appl. Biomater.* **84B**, 301-319.

Lewis, G., Xu, J., Madigan, S., and Towler, M.R. (2007a). Influence of strontia on various properties of Surgical Simplex® P acrylic bone cement and experimental variants. *Acta Biomat.* **3**, 970-979.

Lewis, G., Xu, J., Madigan, S., and Towler, M.R. (2007b). Influence of two changes in the composition of an acrylic bone cement on its handling, thermal, physical, and mechanical properties. *J. Mater. Sci. Mater. Med.* **18**, 1649-1658.

Li, Y.C., Huang, F.M., Lee, S.S., Lin, R.H., Chou, M.Y., and Chang, Y.C. (2008). Protective effects of antioxidants on micronuclei induced by irradiated 9-fluorenone/ N,N-dimethyl-*p*-toluidine in CHO cells. *J. Biomed. Mater. Res. B. Appl. Biomater.* **84**, 58-63.

Lide, D.R., Ed. (1997). *CRC Handbook of Chemistry and Physics*, 78th ed., p. 3-26. CRC Press, Boca Raton, FL.

Linder, L. (1976). Tissue reaction to methyl methacrylate monomer. A comparative study in the rabbit's ear on the toxicity of methyl methacrylate monomer of varying composition. *Acta Orthop. Scand.* **47**, 3-10.

Liso, P.A., Vázquez, B., Rebuelta, M., Hernáez, M.L., Rotger, R., and San Román, J. (1997). Analysis of the leaching and toxicity of new amine activators for the curing of acrylic bone cements and composites. *Biomaterials* **18**, 15-20.

Lovelace Biomedical and Environmental Research Institute (2006). Metabolism and disposition of *N,N*-dimethyl-*p*-toluidine (DMPT) in rats and mice after oral and intravenous administration. Project Report No. 3, Contract No. N01-ES-25483. Submitted to the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

McConnell, E.E., Solleveld, H.A., Swenberg, J.A., and Boorman, G.A. (1986). Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. *JNCI* **76**, 283-289.

MacGregor, J.T., Wehr, C.M., Henika, P.R., and Shelby, M.D. (1990). The *in vivo* erythrocyte micronucleus test: Measurement at steady state increases assay efficiency and permits integration with toxicity studies. *Fundam. Appl. Toxicol.* **14**, 513-522.

Mallinckrodt Baker, Inc. (1996). Material Safety Data Sheet: N,N-Dimethyl-p-toluidine. <http://www.jybaker.com/msds/ d7176.htm> Accessed July 9, 1999.

Mann, H.S., and Whitney, D.R. (1947). On a test of whether one of two random variables is stochastically larger than the other. *Ann. Math. Statist.* **18**, 50-60.

Maronpot, R.R., and Boorman, G.A. (1982). Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. *Toxicol. Pathol.* **10**, 71-80.

Marques, M.M., Mourato, L.L., Amorim, M.T., Santos, M.A., Melchior, W.B., Jr., and Beland, F.A. (1997). Effect of substitution site upon the oxidation potentials of alkylanilines, the mutagencities of N-hydroxyalkylanilines, and the conformations of alkylanaline-DNA adducts. *Chem. Res. Toxicol.* **10**, 1266-1274.

Marsolek, M.R., White, N.C., and Litovitz, T.L. (2010). Inhalant abuse: Monitoring trends by using poison control data, 1993-2008. *Pediatrics* **125**, 906-913.

Masuki, K., Nomura, Y., Bhawal, U.K., Sawajiri, M., Hirata, I., Nahara, Y., and Okazaki, M. (2007). Apoptotic and necrotic influence of dental resin polymerization initiators in human gingival fibroblast cultures. *Dent. Mater. J.* **26**, 861-869.

Miller, E.G., Washington, V.H., Bowles, W.H., and Zimmermann, E.R. (1986). Mutagenic potential of some chemical components of dental materials. *Dent. Mater.* **2**, 163-165.

Miller, J.A., and Miller, E.C. (1977). Ultimate chemical carcinogens as reactive mutagenic electrophiles. In *Origins of Human Cancer* (H.H. Hiatt, J.D. Watson, and J.A. Winsten, Eds.), pp. 605-627. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Misiak, H., and Scheffler, I. (2003). Activator for cyanoacrylate adhesives. United States Patent No. US 6,547,917 B1.

National Cancer Institute (NCI) (1978a). Bioassay of *o*-Anisidine Hydrochloride for Possible Carcinogenicity (CAS No. 134-29-2). Technical Report Series No. 89. NIH Publication No. 78-1339. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Cancer Institute (NCI) (1978b). Bioassay of *p*-Anisidine Hydrochloride for Possible Carcinogenicity (CAS No. 20265-97-8). Technical Report Series No. 116. NIH Publication No. 78-1371. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Institute for Occupational Safety and Health (NIOSH) (1990). National Occupational Exposure Survey (1981-1983), unpublished provisional data as of July 1, 1990. NIOSH, Cincinnati, OH.

National Institute for Occupational Safety and Health (NIOSH) (2007). NIOSH Pocket Guide to Chemical Hazards. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Washington, D.C.

National Toxicology Program (NTP) (1989). Toxicology and Carcinogenesis Studies of Benzofuran (CAS No. 271-89-6) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies). Technical Report Series No. 370. NIH Publication No. 90-2825. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (1992). Toxicology and Carcinogenesis Studies of Ethylene Thiourea (CAS No. 96-45-7) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies). Technical Report Series No. 388. NIH Publication No. 92-2843. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (1993a). Toxicology and Carcinogenesis Studies of *o*-Nitroanisole (CAS No. 91-23-6) in F344 Rats and B6C3F$_1$ Mice (Feed Studies). Technical Report Series No. 416. NIH Publication No. 93-3147. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (1993b). Toxicology and Carcinogenesis Studies of Coumarin (CAS No. 91-64-5) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies). Technical Report Series No. 422. NIH Publication No. 93-3153. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (1995). Toxicology and Carcinogenesis Studies of Methylphenidate Hydrochloride (CAS No. 298-59-9) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies). Technical Report Series No. 439. NIH Publication No. 95-3355. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (1996a). Toxicity and Carcinogenicity Studies of *o*-Nitrotoluene and *o*-Toluidine Hydrochloride (CAS Nos. 88-72-2 and 636-21-5) Administered in Feed to Male F344/N Rats. Toxicity Report Series No. 44. NIH Publication No. 96-3936. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (1996b). Toxicology and Carcinogenesis Studies of 1-Amino-2,4-Dibromoanthraquinone (CAS No. 81-49-2) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies). Technical Report Series No. 383. NIH Publication No. 96-2838. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (1998). Toxicology and Carcinogenesis Studies of Oxazepam (CAS No. 604-75-1) in F344/N Rats (Feed Studies). Technical Report Series No. 468. NIH Publication No. 99-3958. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (2000a). Toxicology and Carcinogenesis Studies of Pyridine (CAS No. 110-86-1) in F344/N Rats, Wistar Rats, and B6C3F$_1$ Mice (Drinking Water Studies). Technical Report Series No. 470. NIH Publication No. 00-3960. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (2000b). Toxicology and Carcinogenesis Studies of Primidone (CAS No. 125-33-7) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies). Technical Report Series No. 476. NIH Publication No. 00-3966. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (2002). Toxicology and Carcinogenesis Studies of *o*-Nitrotoluene (CAS No. 88-72-2) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies). Technical Report Series No. 504. NIH Publication No. 02-4438. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (2004). Toxicity Studies of Butanal Oxime (CAS No. 110-69-0) Administered in Drinking Water and by Gavage to F344/N Rats and B6C3F1 Mice. Toxicity Report Series No. 69. NIH Publication No. 04-4417. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

National Toxicology Program (NTP) (2010). Toxicology and Carcinogenesis Studies of Goldenseal Root Powder (*Hydrastis canadensis*) in F344/N Rats and B6C3F1 Mice (Feed Studies). Technical Report Series No. 562. NIH Publication No. 10-5903. National Institutes of Health, Public Health Service, U.S. Department of Health and Human Services, Research Triangle Park, NC.

Neumark, Y.D., Delva, J., and Anthony, J.C. (1998). The epidemiology of adolescent inhalant drug involvement. *Arch. Pediatr. Adolesc. Med*. **152**, 781-786.

Nomura, Y., Ishibashi, H., Miyahara, M., Shinohara, R., Shiraishi, F., and Arizono, K. (2003). Effects of dental resin metabolites on estrogenic activity *in vitro*. *J. Mater. Sci. Mater. Med*. **14**, 307-310.

Organisation for Economic Co-operation and Development (OECD) (2010). Test No. 487: *In Vitro* Mammalian Cell Micronucleus Test. In *OECD Guidelines for Testing of Chemicals Section 4: Health Effects*. Paris, France.

Pallais, J.C., Mackool, B.T., and Pitman, M.B. (2011). Case records of the Massachusetts General Hospital: Case 7-2011: A 52-year old man with upper respiratory symptoms and low oxygen saturation levels. *N. Engl. J. Med.* **364**, 957-966.

Pereira, S.G., Telo, J.P., and Nunes, T.G. (2008). Towards a controlled photopolymerization of dental dimethacrylate monomers: EPR studies on effects of dilution, filler loading, storage and aging. *J. Mater. Sci. Mater. Med*. **19**, 3135-3144.

Peter, M.G. (1989). Chemical modifications of biopolymers by quinones and quinone methides. *Angew. Chem. Int. Ed. Engl.* **28**, 555-570.

Piegorsch, W.W., and Bailer, A.J. (1997). *Statistics for Environmental Biology and Toxicology*, Section 6.3.2. Chapman and Hall, London.

Plopper, C.G., Suverkropp, C., Morin, D., Nishio, S., and Buckpitt, A. (1992). Relationship of cytochrome P-450 activity to Clara cell cytotoxicity. I. Histopathologic comparison of the respiratory tract of mice, rats and hamsters after parenteral administration of naphthalene. *J. Pharmacol. Exp. Ther.* **261**, 353-363.

Portier, C.J., and Bailer, A.J. (1989). Testing for increased carcinogenicity using a survival-adjusted quantal response test. *Fundam. Appl. Toxicol.* **12**, 731-737.

Portier, C.J., Hedges, J.C., and Hoel, D.G. (1986). Age-specific models of mortality and tumor onset for historical control animals in the National Toxicology Program's carcinogenicity experiments. *Cancer Res.* **46**, 4372-4378.

Potter, J.L., Krill, C.E., Jr., Neal, D., and Kofron, W.G. (1988). Methemoglobinemia due to ingestion of N,N-dimethyl-p-toluidine, a component used in the fabrication of artificial fingernails. *Ann. Emerg. Med.* **17**, 1098-1100.

Rao, G.N. (1996). New diet (NTP-2000) for rats in the National Toxicology Program toxicity and carcinogenicity studies. *Fundam. Appl. Toxicol.* **32**, 102-108.

Rao, G.N. (1997). New nonpurified diet (NTP-2000) for rodents in the National Toxicology Program's toxicology and carcinogenesis studies. *J. Nutr*. **127**, 842s-846s.

Recio, L., Hobbs, C., Caspary, W., and Witt, K.L. (2010). Dose-response assessment of four genotoxic chemicals in a combined mouse and rat micronucleus (MN) and Comet assay protocol. *J. Toxicol. Sci*. **35**, 149-162.

Registry of Toxic Effects of Chemical Substances (RTECS) (2011). N,N-Dimethyl-p-toluidine. National Library of Medicine, Bethesda, MD.

Sadtler Condensed Phase IR Standards Library (March 2, 2004). Spectrum No. SA8303. KnowItAll Informatics Systems, Version 3.005.

Sadtler-Aldrich Library of Condensed Phase IR Standards (2004). Spectrum SA 8303. Sadtler Research Libraries, Philadelphia.

Santosh, V., Ranjith, K., Shenoi, S.D., Sachin, V., and Balachandran, C. (1999). Results of patch testing with dental materials. *Contact Dermatitis* **40**, 50-51.

Sasaki, Y.F., Sekihashi, K., Izumiyama, F., Nishidate, E., Saga, A., Ishida, K., and Tsuda, S. (2000). The comet assay with multiple mouse organs: Comparison of comet assay results and carcinogenicity with 208 chemicals selected from the IARC monographs and U.S. NTP Carcinogenicity Database. *Crit. Rev. Toxicol*. **30**, 629-799.

Schmid, W. (1975). The micronucleus test. *Mutat. Res.* **31**, 9-15.

Shelby, M.D., and Witt, K.L. (1995). Comparison of results from mouse bone marrow chromosome aberration and micronucleus tests. *Environ. Mol. Mutagen.* **25**, 302-313.

Shelby, M.D., Erexson, G.L., Hook, G.J., and Tice, R.R. (1993). Evaluation of a three-exposure mouse bone marrow micronucleus protocol: Results with 49 chemicals. *Environ. Mol. Mutagen.* **21**, 160-179.

Sherratt, A.J., and Damani, L.A. (1989). The metabolism of N,N-dimethylaniline by isolated rat hepatocytes: Identification of a novel N-conjugate. *Xenobiotica* **19**, 379-388.

Shintani, H., Tsuchiya, T., Hata, Y., and Nakamura, A. (1993). Solid phase extraction and HPLC analysis of toxic components eluted from methyl methacrylate dental materials. *J. Anal. Toxicol*. **17**, 73-78.

Shirley, E. (1977). A non-parametric equivalent of Williams' test for contrasting increasing dose levels of a treatment. *Biometrics* **33**, 386-389.

Sigma-Aldrich Internet Catalog (2004). Number D18900-6, July 2, 2004.

Smith, R.P. (1996). Toxic responses of the blood. In *Casarett and Doull's Toxicology: The Basic Science of Poisons*, 5th ed. (M.O. Amdur, J. Doull, and C.D. Klaassen, Eds.), pp. 335-354. McGraw-Hill, New York.

Statistical Data Base System (SDBS) (June 7, 2004). Proton NMR No. 3077HSP-06-320; Carbon-13 NMR No. 3077CDS-03-164. http://www.aist.go.jp/RIODB/SDBS. National Institute of Advanced Industrial Science and Technology (AIST), Japan.

Stea, S., Granchi, D., Zolezzi, C., Ciapetti, G., Visentin, M., Cavedagna, D., and Pizzoferrato, A. (1997). High-performance liquid chromatography assay of *N,N*-dimethyl-*p*-toluidine released from bone cements: Evidence for toxicity. *Biomaterials* **18**, 243-246.

Stolk, J.M., and Smith, R.P. (1966). Species differences in methemoglobin reductase activity. *Biochem. Pharmacol.* **15**, 343-351.

Stout, M.D., Kissling, G.E., Suárez, F.A., Malarkey, D.E., Herbert, R.A., and Bucher, J.R. (2008). Influence of *Helicobacter hepaticus* infection on the chronic toxicity and carcinogenicity of triethanolamine in B6C3F1 mice. *Toxicol. Pathol.* **36**, 783-794.

Straus, D.S. (1981). Somatic mutation, cellular differentiation, and cancer causation. *JNCI* **67**, 233-241.

Takahashi, K., Dinse, G.E., Foley, J.F., Hardisty, J.F., and Maronpot, R.R. (2002). Comparative prevalence, multiplicity, and progression of spontaneous and vinyl carbamate-induced liver lesions in five strains of male mice. *Toxicol. Pathol.* **30**, 599-605.

Taningher, M., Pasquini, R., and Bonatti, S. (1993). Genotoxicity analysis of *N,N*-dimethylaniline and *N,N*-dimethyl-*p*-toluidine. *Environ. Mol. Mutagen.* **21**, 349-356.

Tarone, R.E. (1975). Tests for trend in life table analysis. *Biometrika* **62**, 679-682.

Tennant, R.W., Margolin, B.H., Shelby, M.D., Zeiger, E., Haseman, J.K., Spalding, J., Caspary, W., Resnick, M., Stasiewicz, S., Anderson, B., and Minor, R. (1987). Prediction of chemical carcinogenicity in rodents from in vitro genetic toxicity assays. *Science* **236**, 933-941.

Tice, R.R., Agurell, E., Anderson, D., Burlinson, B., Hartmann, A., Kobayashi, H., Miyamae, Y., Rojas, E., Ryu, J.-C., and Sasaki, Y.F. (2000). Single cell gel/Comet assay: Guidelines for *in vitro* and *in vivo* genetic toxicology testing. *Environ. Mol. Mutagen*. **35**, 206-221.

Timmer, M.D., Ambrose, C.G., and Mikos, A.G. (2003). Evaluation of thermal- and photo-crosslinked biodegradable poly(propylene fumarate)-based networks. *J. Biomed. Mater. Res.* **66A**, 811-818.

Tosti, A., Bardazzi, E., Piancastelli, E., and Brasile, G.P. (1990). Contact stomatitis due to N,N-dimethyl-para-toluidine. *Contact Dermatitis* **22**, 113.

Travlos, G.S., Mahler, J., Ragan, H.A., Chou, B.J., and Bucher, J.R. (1996). Thirteen-week inhalation toxicity of 2- and 4-chloronitrobenzene in F344/N rats and B6C3F1 mice. *Fundam. Appl. Toxicol*. **30**, 75-92.

United States Environmental Protection Agency (USEPA) (2011a). Non-confidential 2006 IUR Records by Chemical, including Manufacturing, Processing and Use Information. CAS No. 99-97-8, Benzenamine, N,N,4-trimethyl. < http://cfpub.epa.gov/iursearch/2006_iur_companyinfo.cfm?chemid=6749&outchem=both > Accessed May 18, 2011.

United States Environmental Protection Agency (USEPA) (2011b). High Production Volume Information System. Benzenamine, N,N,4-trimethyl, CAS No. 99-97-8. <http://iaspub.epa.gov/oppthpv/quicksearch.display?pChem=100729> Accessed May 18, 2011.

Vazquez, B., Levenfeld, B., and San Roman, J. (1998). Role of amine activators on the curing parameters, properties and toxicity of acrylic bone cements. *Polym. Int.* **46**, 241-250.

Verschueren, G.L.A., and Bruynzeel, D.P. (1991). Allergy to N,N-dimethyl-p-toluidine in dental materials. *Contact Dermatitis* **24**, 149.

Verschueren, K., Ed. (1996). *Handbook of Environmental Data on Organic Chemicals*, 3rd ed., pp. 842-843. Van Nostrand Reinhold, New York.

Weisburger, E.K., Russfield, A.B., Homburger, F., Weisburger, J.H., Boger, E., van Dongen, C.G., and Chu, K.C. (1978). Testing of twenty-one environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity. *J. Environ. Pathol. Toxicol.* **2**, 325-356.

Williams, D.A. (1971). A test for differences between treatment means when several dose levels are compared with a zero dose control. *Biometrics* **27**, 103-117.

Williams, D.A. (1972). The comparison of several dose levels with a zero dose control. *Biometrics* **28**, 519-531.

Williams, D.A. (1986). A note on Shirley's nonparametric test for comparing several dose levels with a zero-dose control. *Biometrics* **42**, 183-186.

Winter, K., Pagoria, D., and Geurtsen, W. (2005). The effect of antioxidants on oxidative DNA damage induced by visible-light-irradiated camphorquinone/*N,N*-dimethyl-*p*-toluidine. *Biomaterials* **26**, 5321-5329.

Witt, K.L., Knapton, A., Wehr, C.M., Hook, G.J., Mirsalis, J., Shelby, M.D., and MacGregor, J.T. (2000). Micronucleated erythrocyte frequency in peripheral blood of B6C3F1 mice from short-term, prechronic, and chronic studies of the NTP Carcinogenesis Bioassay Program. *Environ. Mol. Mutagen.* **36**, 163-194.

Witt, K.L., Livanos, E., Kissling, G.E., Torous, D.K., Caspary, W., Tice, R.R., and Recio, L. (2008). Comparison of flow cytometry and microscopy-based methods for measuring micronucleated reticulocyte frequencies in rodents treated with nongenotoxic and genotoxic chemicals. *Mutat. Res*. **649**, 101-113.

Wu, L.-T., Howard, M.O., and Pilowsky, D.J. (2008). Substance abuse disorders among inhalant users: Results from the National Epidemiologic Survey on Alcohol and Related Conditions. *Addict. Behav*. **33**, 968-973.

Zeiger, E., Haseman, J.K., Shelby, M.D., Margolin, B.H., and Tennant, R.W. (1990). Evaluation of four in vitro genetic toxicity tests for predicting rodent carcinogenicity: Confirmation of earlier results with 41 additional chemicals. *Environ. Mol. Mutagen.* **16** (Suppl. 18), 1-14.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., and Mortelmans, K. (1992). Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. *Environ. Mol. Mutagen.* **19** (Suppl. 21), 2-141.

APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR GAVAGE STUDY OF *N*,*N*-DIMETHYL-*p*-TOLUIDINE

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine[a]

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths		1	1	3
Moribund	11	7	11	7
Natural deaths	2	5	7	19
Survivors				
Terminal kill	37	37	31	21
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Carcinoma		1 (2%)		
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma			1 (2%)	1 (2%)
Hepatocellular carcinoma			1 (2%)	6 (12%)
Mesentery	(7)	(5)	(2)	(1)
Pancreas	(50)	(50)	(50)	(50)
Salivary glands	(50)	(50)	(50)	(50)
Sarcoma	1 (2%)			
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(0)	(1)	(0)	(1)
Squamous cell carcinoma				1 (100%)
Squamous cell papilloma		1 (100%)		
Tooth	(1)	(2)	(2)	(0)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Carcinoma, metastatic, uncertain primary site	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			1 (2%)
Osteosarcoma, metastatic, bone		1 (2%)		
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	6 (12%)	2 (4%)	8 (16%)	4 (8%)
Pheochromocytoma complex	1 (2%)	1 (2%)		
Pheochromocytoma malignant	1 (2%)		3 (6%)	1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	2 (4%)	6 (12%)	4 (8%)	1 (2%)
Carcinoma	1 (2%)			
Parathyroid gland	(49)	(49)	(45)	(48)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Endocrine System (continued)				
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	23 (46%)	27 (54%)	18 (36%)	15 (30%)
Pars distalis, carcinoma	1 (2%)	1 (2%)		
Pars intermedia, carcinoma				1 (2%)
Thyroid gland	(50)	(49)	(50)	(49)
Sarcoma, metastatic, salivary glands	1 (2%)			
Bilateral, C-cell, adenoma	1 (2%)		1 (2%)	1 (2%)
C-cell, adenoma	10 (20%)	7 (14%)	7 (14%)	2 (4%)
C-cell, carcinoma		1 (2%)		
Follicular cell, adenoma	1 (2%)		1 (2%)	3 (6%)
Follicular cell, carcinoma		2 (4%)	1 (2%)	2 (4%)
General Body System				
Tissues NOS	(0)	(0)	(2)	(0)
Carcinoma, metastatic, Zymbal's gland			1 (50%)	
Genital System				
Coagulating gland	(1)	(2)	(1)	(0)
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(50)	(50)
Adenoma		1 (2%)	1 (2%)	
Prostate	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Hemangioma	1 (2%)			
Bilateral, interstitial cell, adenoma	29 (58%)	23 (46%)	33 (66%)	25 (50%)
Interstitial cell, adenoma	11 (22%)	13 (26%)	10 (20%)	12 (24%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(4)	(3)	(3)	(7)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)		
Thymus	(50)	(48)	(48)	(47)
Integumentary System				
Mammary gland	(50)	(50)	(49)	(50)
Carcinoma		1 (2%)		
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma	1 (2%)		1 (2%)	1 (2%)
Basal cell carcinoma		1 (2%)	3 (6%)	
Fibroma	5 (10%)	3 (6%)	5 (10%)	3 (6%)
Fibroma, multiple	1 (2%)	1 (2%)		
Keratoacanthoma	3 (6%)	3 (6%)	1 (2%)	2 (4%)
Keratoacanthoma, multiple			1 (2%)	
Lipoma		1 (2%)	1 (2%)	
Liposarcoma			1 (2%)	
Sarcoma		1 (2%)	1 (2%)	
Squamous cell papilloma				1 (2%)
Trichoepithelioma				1 (2%)
Sebaceous gland, adenoma	1 (2%)		2 (4%)	1 (2%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Chondroma			1 (2%)	
Osteosarcoma	1 (2%)	1 (2%)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Astrocytoma malignant			1 (2%)	
Carcinoma, metastatic, pituitary gland	1 (2%)	1 (2%)		1 (2%)
Granular cell tumor malignant			1 (2%)	
Spinal cord	(0)	(1)	(0)	(0)
Osteosarcoma, metastatic, bone		1 (100%)		
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma		1 (2%)	1 (2%)	1 (2%)
Carcinoma, metastatic, tissues NOS			1 (2%)	
Hepatocellular carcinoma, metastatic, liver				2 (4%)
Osteosarcoma, metastatic, bone		1 (2%)		
Pheochromocytoma malignant, metastatic, adrenal medulla	1 (2%)		1 (2%)	1 (2%)
Nose	(50)	(49)	(50)	(49)
Glands, olfactory epithelium, adenoma				1 (2%)
Transitional epithelium, adenoma		3 (6%)	2 (4%)	11 (22%)
Transitional epithelium, carcinoma				2 (4%)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Zymbal's gland	(49)	(50)	(50)	(50)
Adenoma				1 (2%)
Carcinoma			2 (4%)	1 (2%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Oncocytoma benign		1 (2%)		
Renal tubule, adenoma	1 (2%)			
Ureter	(0)	(0)	(0)	(1)
Urinary bladder	(50)	(50)	(50)	(50)
Transitional epithelium, papilloma				1 (2%)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Leukemia mononuclear	14 (28%)	1 (2%)	2 (4%)	
Mesothelioma malignant	1 (2%)	2 (4%)	3 (6%)	1 (2%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]	49	48	46	44
Total primary neoplasms	118	107	119	104
Total animals with benign neoplasms	48	46	46	43
Total benign neoplasms	97	93	100	89
Total animals with malignant neoplasms	19	13	16	15
Total malignant neoplasms	21	14	19	15
Total animals with metastatic neoplasms	4	2	2	4
Total metastatic neoplasms	4	4	3	4
Total animals with malignant neoplasms of uncertain primary site	1			

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	6/50 (12%)	2/50 (4%)	8/50 (16%)	4/50 (8%)
Adjusted rate[b]	13.0%	4.5%	19.2%	10.1%
Terminal rate[c]	6/37 (16%)	2/37 (5%)	7/31 (23%)	1/21 (5%)
First incidence (days)	727 (T)	727 (T)	654	616
Poly-3 test[d]	P=0.531	P=0.140N	P=0.312	P=0.467N
Adrenal Medulla: Malignant Pheochromocytoma				
Overall rate	1/50 (2%)	0/50 (0%)	3/50 (6%)	1/50 (2%)
Adjusted rate	2.2%	0.0%	7.1%	2.5%
Terminal rate	0/37 (0%)	0/37 (0%)	1/31 (3%)	0/21 (0%)
First incidence (days)	688	—[e]	538	601
Poly-3 test	P=0.479	P=0.505N	P=0.275	P=0.725
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma				
Overall rate	8/50 (16%)	3/50 (6%)	11/50 (22%)	5/50 (10%)
Adjusted rate	17.3%	6.7%	25.8%	12.5%
Terminal rate	7/37 (19%)	2/37 (5%)	8/31 (26%)	1/21 (5%)
First incidence (days)	688	721	538	601
Poly-3 test	P=0.552N	P=0.106N	P=0.238	P=0.374N
Liver: Hepatocellular Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	1/50 (2%)	6/50 (12%)
Adjusted rate	0.0%	0.0%	2.4%	14.9%
Terminal rate	0/37 (0%)	0/37 (0%)	1/31 (3%)	2/21 (10%)
First incidence (days)	—	—	727 (T)	612
Poly-3 test	P<0.001	—[f]	P=0.479	P=0.009
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	2/50 (4%)	6/50 (12%)
Adjusted rate	0.0%	0.0%	4.8%	14.9%
Terminal rate	0/37 (0%)	0/37 (0%)	1/31 (3%)	2/21 (10%)
First incidence (days)	—	—	688	612
Poly-3 test	P<0.001	—	P=0.215	P=0.009
Nose: Adenoma				
Overall rate	0/50 (0%)	3/49 (6%)	2/50 (4%)	11/49 (22%)
Adjusted rate	0.0%	6.7%	4.8%	27.5%
Terminal rate	0/37 (0%)	2/37 (5%)	1/31 (3%)	7/21 (33%)
First incidence (days)	—	713	688	582
Poly-3 test	P<0.001	P=0.113	P=0.215	P<0.001
Nose: Adenoma or Carcinoma				
Overall rate	0/50 (0%)	3/49 (6%)	2/50 (4%)	13/49 (27%)
Adjusted rate	0.0%	6.7%	4.8%	32.3%
Terminal rate	0/37 (0%)	2/37 (5%)	1/31 (3%)	7/21 (33%)
First incidence (days)	—	713	688	582
Poly-3 test	P<0.001	P=0.113	P=0.215	P<0.001
Pancreatic Islets: Adenoma				
Overall rate	2/50 (4%)	6/50 (12%)	4/50 (8%)	1/50 (2%)
Adjusted rate	4.4%	13.2%	9.6%	2.6%
Terminal rate	2/37 (5%)	4/37 (11%)	3/31 (10%)	1/21 (5%)
First incidence (days)	727 (T)	601	712	727 (T)
Poly-3 test	P=0.222N	P=0.129	P=0.291	P=0.557N

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	6/50 (12%)	4/50 (8%)	1/50 (2%)
Adjusted rate	6.5%	13.2%	9.6%	2.6%
Terminal rate	3/37 (8%)	4/37 (11%)	3/31 (10%)	1/21 (5%)
First incidence (days)	727 (T)	601	712	727 (T)
Poly-3 test	P=0.162N	P=0.236	P=0.444	P=0.366N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	23/50 (46%)	27/50 (54%)	18/50 (36%)	15/50 (30%)
Adjusted rate	48.6%	57.5%	42.3%	36.7%
Terminal rate	18/37 (49%)	20/37 (54%)	14/31 (45%)	7/21 (33%)
First incidence (days)	610	512	581	496
Poly-3 test	P=0.062N	P=0.251	P=0.351N	P=0.180N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	24/50 (48%)	28/50 (56%)	18/50 (36%)	15/50 (30%)
Adjusted rate	50.7%	59.2%	42.3%	36.7%
Terminal rate	19/37 (51%)	20/37 (54%)	14/31 (45%)	7/21 (33%)
First incidence (days)	610	512	581	496
Poly-3 test	P=0.040N	P=0.264	P=0.279N	P=0.132N
Skin: Keratoacanthoma				
Overall rate	3/50 (6%)	3/50 (6%)	2/50 (4%)	2/50 (4%)
Adjusted rate	6.5%	6.7%	4.8%	5.1%
Terminal rate	3/37 (8%)	3/37 (8%)	1/31 (3%)	1/21 (5%)
First incidence (days)	727 (T)	727 (T)	707	661
Poly-3 test	P=0.473N	P=0.652	P=0.546N	P=0.571N
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	3/50 (6%)	3/50 (6%)	2/50 (4%)	3/50 (6%)
Adjusted rate	6.5%	6.7%	4.8%	7.6%
Terminal rate	3/37 (8%)	3/37 (8%)	1/31 (3%)	2/21 (10%)
First incidence (days)	727 (T)	727 (T)	707	661
Poly-3 test	P=0.519	P=0.652	P=0.546N	P=0.588
Skin: Basal Cell Carcinoma				
Overall rate	0/50 (0%)	1/50 (2%)	3/50 (6%)	0/50 (0%)
Adjusted rate	0.0%	2.2%	7.2%	0.0%
Terminal rate	0/37 (0%)	1/37 (3%)	3/31 (10%)	0/21 (0%)
First incidence (days)	—	727 (T)	727 (T)	—
Poly-3 test	P=0.578N	P=0.496	P=0.101	—
Skin: Trichoepithelioma, Basal Cell Adenoma, or Basal Cell Carcinoma				
Overall rate	1/50 (2%)	1/50 (2%)	4/50 (8%)	2/50 (4%)
Adjusted rate	2.2%	2.2%	9.7%	5.1%
Terminal rate	1/37 (3%)	1/37 (3%)	4/31 (13%)	1/21 (5%)
First incidence (days)	727 (T)	727 (T)	727 (T)	616
Poly-3 test	P=0.326	P=0.756	P=0.148	P=0.447
Skin: Squamous Cell Papilloma, Keratoacanthoma, Trichoepithelioma, Basal Cell Adenoma, or Basal Cell Carcinoma				
Overall rate	4/50 (8%)	4/50 (8%)	6/50 (12%)	4/50 (8%)
Adjusted rate	8.7%	8.9%	14.5%	10.1%
Terminal rate	4/37 (11%)	4/37 (11%)	5/31 (16%)	2/21 (10%)
First incidence (days)	727 (T)	727 (T)	707	616
Poly-3 test	P=0.484	P=0.631	P=0.307	P=0.560

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Skin: Fibroma				
Overall rate	6/50 (12%)	4/50 (8%)	5/50 (10%)	3/50 (6%)
Adjusted rate	13.0%	8.9%	12.0%	7.6%
Terminal rate	5/37 (14%)	4/37 (11%)	4/31 (13%)	1/21 (5%)
First incidence (days)	645	727 (T)	612	645
Poly-3 test	P=0.339N	P=0.388N	P=0.571N	P=0.327N
Skin: Fibroma or Sarcoma				
Overall rate	6/50 (12%)	5/50 (10%)	6/50 (12%)	3/50 (6%)
Adjusted rate	13.0%	11.1%	14.2%	7.6%
Terminal rate	5/37 (14%)	4/37 (11%)	4/31 (13%)	1/21 (5%)
First incidence (days)	645	695	612	645
Poly-3 test	P=0.301N	P=0.518N	P=0.554	P=0.327N
Testes: Adenoma				
Overall rate	40/50 (80%)	36/50 (72%)	43/50 (86%)	37/50 (74%)
Adjusted rate	84.2%	79.4%	94.6%	84.8%
Terminal rate	34/37 (92%)	32/37 (87%)	29/31 (94%)	19/21 (91%)
First incidence (days)	609	695	538	384
Poly-3 test	P=0.418	P=0.363N	P=0.083	P=0.591
Thyroid Gland (C-cell): Adenoma				
Overall rate	11/50 (22%)	7/49 (14%)	8/50 (16%)	3/49 (6%)
Adjusted rate	23.6%	15.7%	19.0%	7.7%
Terminal rate	8/37 (22%)	7/37 (19%)	5/31 (16%)	2/21 (10%)
First incidence (days)	612	727 (T)	616	669
Poly-3 test	P=0.057N	P=0.248N	P=0.395N	P=0.044N
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	11/50 (22%)	8/49 (16%)	8/50 (16%)	3/49 (6%)
Adjusted rate	23.6%	17.9%	19.0%	7.7%
Terminal rate	8/37 (22%)	8/37 (22%)	5/31 (16%)	2/21 (10%)
First incidence (days)	612	727 (T)	616	669
Poly-3 test	P=0.047N	P=0.344N	P=0.395N	P=0.044N
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	1/50 (2%)	0/49 (0%)	1/50 (2%)	3/49 (6%)
Adjusted rate	2.2%	0.0%	2.4%	7.7%
Terminal rate	1/37 (3%)	0/37 (0%)	0/31 (0%)	3/21 (14%)
First incidence (days)	727 (T)	—	680	727 (T)
Poly-3 test	P=0.060	P=0.506N	P=0.738	P=0.248
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	1/50 (2%)	2/49 (4%)	2/50 (4%)	4/49 (8%)
Adjusted rate	2.2%	4.5%	4.8%	10.3%
Terminal rate	1/37 (3%)	2/37 (5%)	1/31 (3%)	4/21 (19%)
First incidence (days)	727 (T)	727 (T)	680	727 (T)
Poly-3 test	P=0.088	P=0.489	P=0.465	P=0.132
All Organs: Mononuclear Cell Leukemia				
Overall rate	14/50 (28%)	1/50 (2%)	2/50 (4%)	0/50 (0%)
Adjusted rate	28.7%	2.2%	4.8%	0.0%
Terminal rate	5/37 (14%)	0/37 (0%)	1/31 (3%)	0/21 (0%)
First incidence (days)	337	624	626	—
Poly-3 test	P<0.001N	P<0.001N	P=0.003N	P<0.001N

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
All Organs: Malignant Mesothelioma				
Overall rate	1/50 (2%)	2/50 (4%)	3/50 (6%)	1/50 (2%)
Adjusted rate	2.2%	4.5%	7.1%	2.6%
Terminal rate	1/37 (3%)	2/37 (5%)	1/31 (3%)	1/21 (5%)
First incidence (days)	727 (T)	727 (T)	626	727 (T)
Poly-3 test	P=0.588N	P=0.492	P=0.274	P=0.723
All Organs: Benign Neoplasms				
Overall rate	48/50 (96%)	46/50 (92%)	46/50 (92%)	43/50 (86%)
Adjusted rate	98.2%	97.7%	99.8%	95.0%
Terminal rate	37/37 (100%)	37/37 (100%)	31/31 (100%)	21/21 (100%)
First incidence (days)	609	512	519	384
Poly-3 test	P=0.173N	P=0.747N	P=0.626	P=0.348N
All Organs: Malignant Neoplasms				
Overall rate	20/50 (40%)	13/50 (26%)	16/50 (32%)	15/50 (30%)
Adjusted rate	40.9%	28.0%	36.3%	36.3%
Terminal rate	10/37 (27%)	8/37 (22%)	8/31 (26%)	7/21 (33%)
First incidence (days)	337	324	538	601
Poly-3 test	P=0.535	P=0.134N	P=0.407N	P=0.412N
All Organs: Benign or Malignant Neoplasms				
Overall rate	49/50 (98%)	48/50 (96%)	46/50 (92%)	44/50 (88%)
Adjusted rate	98.5%	99.2%	99.8%	96.4%
Terminal rate	37/37 (100%)	37/37 (100%)	31/31 (100%)	21/21 (100%)
First incidence (days)	337	324	519	384
Poly-3 test	P=0.210N	P=0.772	P=0.677	P=0.501N

(T) Terminal kill

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal medulla, liver, nose, pancreatic islets, pituitary gland, testes, and thyroid gland; for other tissues, denominator is number of animals necropsied.

[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in a dose group is indicated by **N**.

[e] Not applicable; no neoplasms in animal group

[f] Value of statistic cannot be computed.

TABLE A3a
Historical Incidence of Hepatocellular Neoplasms in Control Male F344/N Rats[a]

Study (Study Start)	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Corn Oil Gavage Studies			
N,N-Dimethyl-*p*-toluidine (October 2004)	0/50	0/50	0/50
Ginkgo biloba extract (March 2005)	0/50	0/50	0/50
Isoeugenol (April 2002)	1/50	0/50	1/50
Kava kava extract (August 2004)	1/49	0/49	1/49
β-Myrcene (March 2002)	0/50	0/50	0/50
Pulegone (April 2003)	1/50	0/50	1/50
Total (%)	3/299 (1.0%)	0/299	3/299 (1.0%)
Mean ± standard deviation	1.0% ± 1.1%		1.0% ± 1.1%
Range	0%-2%		0%-2%
Overall Historical Incidence: All Routes			
Total (%)	18/1,249 (1.4%)	5/1,249 (0.4%)	23/1,249 (1.8%)
Mean ± standard deviation	1.4% ± 1.9%	0.4% ± 1.0%	1.8% ± 1.9%
Range	0%-6%	0%-4%	0%-6%

[a] Data as of May 2011

TABLE A3b
Historical Incidence of Adenoma of the Nose in Control Male F344/N Rats[a]

Study (Study Start)	Incidence in Controls
Historical Incidence: Corn Oil Gavage Studies	
N,N-Dimethyl-*p*-toluidine (October 2004)	0/50
Ginkgo biloba extract (March 2005)	0/50
Isoeugenol (April 2002)	0/50
Kava kava extract (August 2004)	0/49
β-Myrcene (March 2002)	0/50
Pulegone (April 2003)	0/50
Total	0/299
Overall Historical Incidence: All Routes	
Total	0/1,248

[a] Data as of May 2011

TABLE A3c
Historical Incidence of Follicular Cell Neoplasms of the Thyroid Gland in Control Male F344/N Rats[a]

Study (Study Start)	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Corn Oil Gavage Studies			
N,*N*-Dimethyl-*p*-toluidine (October 2004)	1/50	0/50	1/50
Ginkgo biloba extract (March 2005)	2/50	0/50	2/50
Isoeugenol (April 2002)	1/50	1/50	2/50
Kava kava extract (August 2004)	1/49	0/49	1/49
β-Myrcene (March 2002)	1/50	2/50	3/50
Pulegone (April 2003)	0/50	0/50	0/50
Total (%)	6/299 (2.0%)	3/299 (1.0%)	9/299 (3.0%)
Mean ± standard deviation	2.0% ± 1.3%	1.0% ± 1.7%	3.0% ± 2.1%
Range	0%-4%	0%-4%	0%-6%
Overall Historical Incidence: All Routes			
Total (%)	13/1,239 (1.1%)	10/1,239 (0.8%)	23/1,239 (1.9%)
Mean ± standard deviation	1.0% ± 1.7%	0.8% ± 1.5%	1.9% ± 2.2%
Range	0%-6%	0%-4%	0%-6%

[a] Data as of May 2011

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine[a]

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths		1	1	3
Moribund	11	7	11	7
Natural deaths	2	5	7	19
Survivors				
Terminal kill	37	37	31	21
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Foreign body			1 (2%)	
Inflammation				1 (2%)
Perforation			1 (2%)	2 (4%)
Periesophageal tissue, inflammation				1 (2%)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Parasite metazoan	1 (2%)	2 (4%)	1 (2%)	
Intestine large, rectum	(50)	(50)	(50)	(50)
Parasite metazoan	3 (6%)	5 (10%)	2 (4%)	3 (6%)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Inflammation, chronic active			1 (2%)	
Intestine small, ileum	(50)	(50)	(50)	(50)
Parasite metazoan			1 (2%)	
Intestine small, jejunum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)			
Basophilic focus	28 (56%)	6 (12%)		3 (6%)
Clear cell focus	30 (60%)	36 (72%)	26 (52%)	35 (70%)
Congestion	1 (2%)			
Degeneration, cystic	4 (8%)	10 (20%)	9 (18%)	17 (34%)
Eosinophilic focus	11 (22%)	21 (42%)	21 (42%)	29 (58%)
Fatty change, focal	6 (12%)	2 (4%)	3 (6%)	9 (18%)
Fatty change, diffuse	1 (2%)	8 (16%)	5 (10%)	5 (10%)
Hematopoietic cell proliferation	1 (2%)		1 (2%)	1 (2%)
Hemorrhage	1 (2%)			
Hepatodiaphragmatic nodule	8 (16%)	1 (2%)		3 (6%)
Inflammation	40 (80%)	46 (92%)	42 (84%)	44 (88%)
Mixed cell focus	18 (36%)	17 (34%)	17 (34%)	35 (70%)
Vacuolization cytoplasmic		1 (2%)	3 (6%)	1 (2%)
Bile duct, cyst		1 (2%)	3 (6%)	
Bile duct, fibrosis	21 (42%)	27 (54%)	41 (82%)	42 (84%)
Bile duct, hyperplasia	40 (80%)	42 (84%)	44 (88%)	44 (88%)
Hepatocyte, hypertrophy			6 (12%)	31 (62%)
Hepatocyte, necrosis	2 (4%)		2 (4%)	1 (2%)
Oval cell, hyperplasia			2 (4%)	2 (4%)
Mesentery	(7)	(5)	(2)	(1)
Fat, necrosis	7 (100%)	5 (100%)	1 (50%)	1 (100%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Alimentary System (continued)				
Pancreas	(50)	(50)	(50)	(50)
Basophilic focus	1 (2%)			
Cyst	2 (4%)	4 (8%)	6 (12%)	2 (4%)
Hyperplasia		1 (2%)		
Infiltration cellular, mononuclear cell	16 (32%)	14 (28%)	16 (32%)	20 (40%)
Lipomatosis			1 (2%)	
Metaplasia, hepatocyte				1 (2%)
Acinus, atrophy	21 (42%)	20 (40%)	17 (34%)	12 (24%)
Acinus, hyperplasia	2 (4%)	2 (4%)	1 (2%)	
Salivary glands	(50)	(50)	(50)	(50)
Infiltration cellular		1 (2%)		
Stomach, forestomach	(50)	(50)	(50)	(50)
Edema		1 (2%)		
Erosion	1 (2%)			
Hyperplasia, squamous		3 (6%)	5 (10%)	11 (22%)
Inflammation	1 (2%)	5 (10%)	5 (10%)	7 (14%)
Ulcer		2 (4%)	5 (10%)	6 (12%)
Stomach, glandular	(50)	(50)	(50)	(50)
Erosion	1 (2%)			
Inflammation		1 (2%)	2 (4%)	1 (2%)
Mineralization				2 (4%)
Necrosis				1 (2%)
Ulcer		1 (2%)	2 (4%)	
Tongue	(0)	(1)	(0)	(1)
Tooth	(1)	(2)	(2)	(0)
Dysplasia			1 (50%)	
Peridontal tissue, inflammation	1 (100%)	2 (100%)	1 (50%)	
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Aorta, mineralization				1 (2%)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	46 (92%)	50 (100%)	49 (98%)	48 (96%)
Mineralization				3 (6%)
Pigmentation	1 (2%)			
Thrombosis			1 (2%)	2 (4%)
Artery, inflammation		1 (2%)		
Pericardium, inflammation				1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Angiectasis	13 (26%)	9 (18%)	7 (14%)	4 (8%)
Hyperplasia	17 (34%)	21 (42%)	10 (20%)	8 (16%)
Hypertrophy	9 (18%)	6 (12%)	6 (12%)	7 (14%)
Necrosis				1 (2%)
Vacuolization cytoplasmic	31 (62%)	31 (62%)	26 (52%)	28 (56%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	18 (36%)	15 (30%)	12 (24%)	18 (36%)
Infiltration cellular, lymphocyte		1 (2%)		
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	2 (4%)	1 (2%)	
Parathyroid gland	(49)	(49)	(45)	(48)
Cyst		1 (2%)		
Hyperplasia, focal	2 (4%)			
Hyperplasia, diffuse	1 (2%)		2 (4%)	5 (10%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Endocrine System (continued)				
Pituitary gland	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)		
Cyst	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Pars distalis, cyst			1 (2%)	
Pars distalis, hemorrhage	1 (2%)			
Pars distalis, hyperplasia	15 (30%)	18 (36%)	15 (30%)	18 (36%)
Thyroid gland	(50)	(49)	(50)	(49)
C-cell, hyperplasia	14 (28%)	20 (41%)	14 (28%)	5 (10%)
Follicle, cyst		1 (2%)		
Follicular cell, hyperplasia			1 (2%)	
General Body System				
Tissue NOS	(0)	(0)	(2)	(0)
Genital System				
Coagulating gland	(1)	(2)	(1)	(0)
Inflammation	1 (100%)	2 (100%)		
Epithelium, hyperplasia	1 (100%)	1 (50%)		
Epididymis	(50)	(50)	(50)	(50)
Atypia cellular		1 (2%)		
Inflammation		1 (2%)	2 (4%)	2 (4%)
Preputial gland	(50)	(50)	(50)	(50)
Cyst				1 (2%)
Hyperplasia	1 (2%)			1 (2%)
Inflammation	49 (98%)	49 (98%)	43 (86%)	45 (90%)
Prostate	(50)	(50)	(50)	(50)
Inflammation	23 (46%)	28 (56%)	18 (36%)	16 (32%)
Pigmentation				1 (2%)
Epithelium, hyperplasia	2 (4%)	6 (12%)	2 (4%)	3 (6%)
Seminal vesicle	(50)	(50)	(50)	(50)
Inflammation	1 (2%)	1 (2%)		1 (2%)
Epithelium, hyperplasia		1 (2%)		1 (2%)
Testes	(50)	(50)	(50)	(50)
Cyst		1 (2%)		
Mineralization	1 (2%)		1 (2%)	1 (2%)
Interstitial cell, hyperplasia	16 (32%)	16 (32%)	6 (12%)	12 (24%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemorrhage			1 (2%)	4 (8%)
Hyperplasia	17 (34%)	13 (26%)	28 (56%)	50 (100%)
Myelofibrosis			1 (2%)	
Necrosis	1 (2%)			
Lymph node	(4)	(3)	(3)	(7)
Deep cervical, hyperplasia, plasma cell	1 (25%)			
Mediastinal, ectasia		2 (67%)	2 (67%)	3 (43%)
Mediastinal, hemorrhage		1 (33%)		
Mediastinal, hyperplasia, lymphoid			1 (33%)	3 (43%)
Mediastinal, hyperplasia, plasma cell		1 (33%)		1 (14%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Hematopoietic System (continued)				
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Atrophy		1 (2%)		
Ectasia				2 (4%)
Hyperplasia, lymphoid	2 (4%)	1 (2%)		1 (2%)
Hyperplasia, plasma cell				1 (2%)
Infiltration cellular, histiocyte	21 (42%)	23 (46%)	30 (60%)	34 (68%)
Spleen	(50)	(50)	(50)	(50)
Congestion	1 (2%)			39 (78%)
Hematopoietic cell proliferation	34 (68%)	44 (88%)	42 (84%)	44 (88%)
Inflammation, suppurative				2 (4%)
Pigmentation	36 (72%)	48 (96%)	47 (94%)	48 (96%)
Capsule, fibrosis	1 (2%)		2 (4%)	46 (92%)
Capsule, hemorrhage		1 (2%)		
Capsule, hypertrophy, mesothelium		1 (2%)	3 (6%)	39 (78%)
Lymphoid follicle, atrophy		5 (10%)	2 (4%)	19 (38%)
Red pulp, atrophy			1 (2%)	8 (16%)
Thymus	(50)	(48)	(48)	(47)
Atrophy	44 (88%)	46 (96%)	44 (92%)	44 (94%)
Hyperplasia, lymphoid				1 (2%)
Integumentary System				
Mammary gland	(50)	(50)	(49)	(50)
Cyst				1 (2%)
Hyperplasia			2 (4%)	
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion				1 (2%)
Inflammation				1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Demyelination	1 (2%)			
Hemorrhage	1 (2%)			
Spinal cord	(0)	(1)	(0)	(0)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion			1 (2%)	3 (6%)
Foreign body		1 (2%)		
Inflammation	2 (4%)	5 (10%)	6 (12%)	1 (2%)
Mineralization				1 (2%)
Alveolar epithelium, hyperplasia	8 (16%)	9 (18%)	6 (12%)	6 (12%)
Alveolus, foreign body			1 (2%)	
Alveolus, infiltration cellular, histiocyte	14 (28%)	2 (4%)	5 (10%)	11 (22%)
Alveolus, inflammation, suppurative				1 (2%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Respiratory System (continued)				
Nose	(50)	(49)	(50)	(49)
Foreign body	13 (26%)	17 (35%)	11 (22%)	9 (18%)
Inflammation	35 (70%)	40 (82%)	38 (76%)	48 (98%)
Glands, olfactory epithelium, dilatation			3 (6%)	49 (100%)
Glands, olfactory epithelium, hyperplasia		2 (4%)		48 (98%)
Glands, olfactory epithelium, metaplasia				38 (78%)
Glands, olfactory epithelium, necrosis				22 (45%)
Glands, respiratory epithelium, dilatation	13 (26%)	15 (31%)	19 (38%)	48 (98%)
Glands, respiratory epithelium, hyperplasia		8 (16%)	8 (16%)	41 (84%)
Glands, respiratory epithelium, metaplasia, respiratory	29 (58%)	39 (80%)	39 (78%)	47 (96%)
Glands, transitional epithelium, dilatation			5 (10%)	3 (6%)
Glands, transitional epithelium, hyperplasia		1 (2%)	24 (48%)	40 (82%)
Nerve, atrophy				15 (31%)
Olfactory epithelium, accumulation, hyaline droplet	49 (98%)	44 (90%)	40 (80%)	
Olfactory epithelium, degeneration			1 (2%)	47 (96%)
Olfactory epithelium, hyperplasia, basal cell		1 (2%)	2 (4%)	38 (78%)
Olfactory epithelium, metaplasia, respiratory	4 (8%)	9 (18%)	9 (18%)	40 (82%)
Respiratory epithelium, accumulation, hyaline droplet	42 (84%)	35 (71%)	30 (60%)	8 (16%)
Respiratory epithelium, hyperplasia	15 (30%)	29 (59%)	32 (64%)	49 (100%)
Respiratory epithelium, ulcer				1 (2%)
Squamous epithelium, cyst		1 (2%)		
Transitional epithelium, hyperplasia	1 (2%)	1 (2%)	11 (22%)	46 (94%)
Trachea	(50)	(50)	(50)	(50)
Inflammation	3 (6%)	1 (2%)	1 (2%)	3 (6%)
Perforation				1 (2%)
Peritracheal tissue, inflammation				2 (4%)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Dysplasia		1 (2%)		
Inflammation		1 (2%)		
Cornea, hyperplasia		1 (2%)		
Cornea, inflammation	1 (2%)	1 (2%)		
Lens, degeneration		1 (2%)		1 (2%)
Retina, atrophy		1 (2%)	1 (2%)	3 (6%)
Harderian gland	(50)	(50)	(50)	(50)
Infiltration cellular, lymphoid		1 (2%)		
Inflammation	3 (6%)	6 (12%)		10 (20%)
Zymbal's gland	(49)	(50)	(50)	(50)
Hyperplasia	1 (2%)		1 (2%)	

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet	2 (4%)	1 (2%)		
Cyst			1 (2%)	1 (2%)
Mineralization	44 (88%)	37 (74%)	38 (76%)	49 (98%)
Nephropathy	49 (98%)	49 (98%)	48 (96%)	49 (98%)
Pigmentation	24 (48%)	46 (92%)	37 (74%)	44 (88%)
Papilla, necrosis		2 (4%)		
Pelvis, dilatation				3 (6%)
Pelvis, inflammation		3 (6%)	1 (2%)	
Pelvis, transitional epithelium, hyperplasia	1 (2%)	2 (4%)	6 (12%)	5 (10%)
Ureter	(0)	(0)	(0)	(1)
Inflammation				1 (100%)
Urinary bladder	(50)	(50)	(50)	(50)
Hemorrhage		1 (2%)		2 (4%)
Inflammation		2 (4%)	2 (4%)	2 (4%)
Ulcer		1 (2%)		
Transitional epithelium, necrosis				1 (2%)

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR GAVAGE STUDY OF *N*,*N*-DIMETHYL-*p*-TOLUIDINE

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine[a]

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death		1		1
Moribund	14	3	8	8
Natural deaths	3	4	9	18
Survivors				
Terminal kill	33	42	33	23
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Leiomyosarcoma	1 (2%)			
Liver	(50)	(50)	(50)	(49)
Hepatocellular adenoma		1 (2%)	1 (2%)	3 (6%)
Hepatocellular carcinoma				3 (6%)
Hepatocellular carcinoma, multiple				1 (2%)
Mesentery	(8)	(9)	(9)	(3)
Pancreas	(50)	(50)	(50)	(50)
Salivary glands	(50)	(50)	(50)	(48)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)	(50)
Neural crest tumor, metastatic, ear				1 (2%)
Tongue	(1)	(0)	(0)	(2)
Squamous cell carcinoma				1 (50%)
Squamous cell papilloma	1 (100)%)			1 (50%)
Tooth	(0)	(1)	(0)	(0)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(49)
Adenoma	3 (6%)	3 (6%)	1 (2%)	2 (4%)
Adrenal medulla	(50)	(50)	(50)	(49)
Pheochromocytoma benign	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Pheochromocytoma malignant			1 (2%)	
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	
Carcinoma			1 (2%)	
Parathyroid gland	(50)	(50)	(50)	(46)
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	31 (62%)	29 (58%)	30 (60%)	20 (40%)
Thyroid gland	(49)	(47)	(47)	(45)
C-cell, adenoma	9 (18%)	3 (6%)	5 (11%)	2 (4%)
C-cell, carcinoma			1 (2%)	
Follicular cell, adenoma	1 (2%)	1 (2%)	2 (4%)	

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
General Body System				
None				
Genital System				
Clitoral gland	(50)	(49)	(50)	(50)
Adenoma	5 (10%)	3 (6%)	7 (14%)	2 (4%)
Adenoma, multiple			1 (2%)	
Carcinoma		2 (4%)	2 (4%)	
Ovary	(50)	(50)	(50)	(50)
Granulosa cell tumor benign		1 (2%)		
Granulosa cell tumor malignant		2 (4%)		1 (2%)
Bilateral, fibrosarcoma	1 (2%)			
Uterus	(50)	(50)	(50)	(50)
Adenocarcinoma		1 (2%)		
Adenoma			1 (2%)	
Polyp stromal	3 (6%)	9 (18%)	3 (6%)	8 (16%)
Polyp stromal, multiple			1 (2%)	
Sarcoma stromal			1 (2%)	
Vagina	(1)	(1)	(0)	(0)
Polyp		1 (100%)		
Schwannoma malignant	1 (100%)			
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(2)	(1)	(2)	(1)
Deep cervical, sarcoma, metastatic, skin			1 (50%)	
Mediastinal, neural crest tumor, metastatic, ear				1 (100%)
Lymph node, mesenteric	(50)	(49)	(50)	(49)
Spleen	(50)	(50)	(50)	(50)
Thymus	(47)	(50)	(50)	(48)
Thymoma benign			1 (2%)	
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Carcinoma		1 (2%)	2 (4%)	
Carcinoma, multiple	1 (2%)			
Fibroadenoma	17 (34%)	11 (22%)	19 (38%)	11 (22%)
Fibroadenoma, multiple	12 (24%)	15 (30%)	7 (14%)	
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma		1 (2%)		
Basal cell carcinoma	1 (2%)		1 (2%)	1 (2%)
Fibroma	1 (2%)	4 (8%)	1 (2%)	
Fibrosarcoma	2 (4%)			
Fibrosarcoma, multiple	1 (2%)			
Keratoacanthoma	1 (2%)	1 (2%)		1 (2%)
Lipoma			1 (2%)	
Liposarcoma	1 (2%)		1 (2%)	
Sarcoma			1 (2%)	1 (2%)
Schwannoma malignant		1 (2%)	1 (2%)	
Squamous cell papilloma				1 (2%)
Trichoepithelioma		1 (2%)		

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Chordoma				1 (2%)
Osteosarcoma				2 (4%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Oligodendroglioma malignant				1 (2%)
Peripheral nerve	(0)	(0)	(1)	(0)
Spinal cord	(0)	(0)	(1)	(0)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)			1 (2%)
Fibrosarcoma, metastatic, skin	1 (2%)			
Neural creast tumor, metastatic, ear				1 (2%)
Osteosarcoma, metastatic, bone				1 (2%)
Sarcoma, metastatic, skin			1 (2%)	
Nose	(50)	(49)	(50)	(49)
Transitional epithelium, adenoma		1 (2%)		2 (4%)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Ear	(1)	(0)	(0)	(1)
Neural crest tumor	1 (100%)			1 (100%)
Eye	(50)	(50)	(50)	(50)
Melanoma benign	1 (2%)			
Harderian gland	(50)	(50)	(50)	(50)
Lacrimal gland	(0)	(0)	(0)	(1)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Carcinoma			1 (2%)	
Lipoma			1 (2%)	
Urinary bladder	(50)	(50)	(50)	(50)
Transitional epithelium, papilloma	1 (2%)			
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Leukemia mononuclear	15 (30%)	2 (4%)	1 (2%)	1 (2%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]	49	46	43	36
Total primary neoplasms	114	96	98	69
Total animals with benign neoplasms	46	44	42	33
Total benign neoplasms	89	87	84	55
Total animals with malignant neoplasms	23	9	12	11
Total malignant neoplasms	24	9	14	13
Total animals with metastatic neoplasms	1		1	2
Total metastatic neoplasms	1		2	4
Total animals with uncertain neoplasms-benign or malignant	1			1
Total uncertain neoplasms	1			1

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Adrenal Cortex: Adenoma				
Overall rate[a]	3/50 (6%)	3/50 (6%)	1/50 (2%)	2/49 (4%)
Adjusted rate[b]	6.6%	6.4%	2.2%	5.2%
Terminal rate[c]	3/33 (9%)	3/42 (7%)	0/33 (0%)	1/23 (4%)
First incidence (days)	728 (T)	728 (T)	613	682
Poly-3 test[d]	P=0.473N	P=0.652N	P=0.299N	P=0.575N
Clitoral Gland: Adenoma				
Overall rate	5/50 (10%)	3/49 (6%)	8/50 (16%)	2/50 (4%)
Adjusted rate	10.8%	6.6%	17.2%	5.1%
Terminal rate	3/33 (9%)	3/41 (7%)	5/33 (15%)	1/23 (4%)
First incidence (days)	646	728 (T)	659	714
Poly-3 test	P=0.344N	P=0.362N	P=0.280	P=0.286N
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	5/50 (10%)	5/49 (10%)	10/50 (20%)	2/50 (4%)
Adjusted rate	10.8%	10.9%	21.3%	5.1%
Terminal rate	3/33 (9%)	5/41 (12%)	6/33 (18%)	1/23 (4%)
First incidence (days)	646	728 (T)	611	714
Poly-3 test	P=0.267N	P=0.624	P=0.136	P=0.286N
Liver: Hepatocellular Adenoma				
Overall rate	0/50 (0%)	1/50 (2%)	1/50 (2%)	3/49 (6%)
Adjusted rate	0.0%	2.1%	2.2%	7.8%
Terminal rate	0/33 (0%)	1/42 (2%)	1/33 (3%)	2/23 (9%)
First incidence (days)	—[e]	728 (T)	728 (T)	720
Poly-3 test	P=0.044	P=0.504	P=0.502	P=0.091
Liver: Hepatocellular Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	4/49 (8%)
Adjusted rate	0.0%	0.0%	0.0%	10.4%
Terminal rate	0/33 (0%)	0/42 (0%)	0/33 (0%)	4/23 (17%)
First incidence (days)	—	—	—	728 (T)
Poly-3 test	P<0.001	—[f]	—	P=0.041
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	0/50 (0%)	1/50 (2%)	1/50 (2%)	7/49 (14%)
Adjusted rate	0.0%	2.1%	2.2%	18.1%
Terminal rate	0/33 (0%)	1/42 (2%)	1/33 (3%)	6/23 (26%)
First incidence (days)	—	728 (T)	728 (T)	720
Poly-3 test	P<0.001	P=0.504	P=0.502	P=0.003
Mammary Gland: Fibroadenoma				
Overall rate	29/50 (58%)	26/50 (52%)	26/50 (52%)	11/50 (22%)
Adjusted rate	60.4%	55.4%	54.5%	27.6%
Terminal rate	18/33 (55%)	24/42 (57%)	17/33 (52%)	9/23 (39%)
First incidence (days)	625	688	646	612
Poly-3 test	P<0.001N	P=0.385N	P=0.349N	P<0.001N
Mammary Gland: Fibroadenoma or Carcinoma				
Overall rate	30/50 (60%)	27/50 (54%)	26/50 (52%)	11/50 (22%)
Adjusted rate	61.9%	57.0%	54.5%	27.6%
Terminal rate	18/33 (55%)	24/42 (57%)	17/33 (52%)	9/23 (39%)
First incidence (days)	574	611	646	612
Poly-3 test	P<0.001N	P=0.392N	P=0.297N	P<0.001N

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Ovary: Benign or Malignant Granulosa Cell Tumor				
Overall rate	0/50 (0%)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	0.0%	6.4%	0.0%	2.5%
Terminal rate	0/33 (0%)	3/42 (7%)	0/33 (0%)	1/23 (4%)
First incidence (days)	—	728 (T)	—	728 (T)
Poly-3 test	P=0.630N	P=0.123	—	P=0.470
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	31/50 (62%)	29/50 (58%)	30/50 (60%)	20/50 (40%)
Adjusted rate	63.5%	60.5%	61.8%	47.8%
Terminal rate	19/33 (58%)	24/42 (57%)	17/33 (52%)	9/23 (39%)
First incidence (days)	547	574	604	471
Poly-3 test	P=0.079N	P=0.462N	P=0.514N	P=0.095N
Skin: Squamous Cell Papilloma, Keratoacanthoma, Trichoepithelioma, Basal Cell Adenoma, or Basal Cell Carcinoma				
Overall rate	2/50 (4%)	3/50 (6%)	1/50 (2%)	3/50 (6%)
Adjusted rate	4.4%	6.4%	2.2%	7.6%
Terminal rate	1/33 (3%)	2/42 (5%)	1/33 (3%)	3/23 (13%)
First incidence (days)	701	574	728 (T)	728 (T)
Poly-3 test	P=0.404	P=0.514	P=0.498N	P=0.431
Skin: Fibroma				
Overall rate	1/50 (2%)	4/50 (8%)	1/50 (2%)	0/50 (0%)
Adjusted rate	2.2%	8.5%	2.2%	0.0%
Terminal rate	1/33 (3%)	3/42 (7%)	1/33 (3%)	0/23 (0%)
First incidence (days)	728 (T)	611	728 (T)	—
Poly-3 test	P=0.151N	P=0.188	P=0.759N	P=0.530N
Skin: Fibrosarcoma				
Overall rate	3/50 (6%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
Adjusted rate	6.6%	0.0%	0.0%	0.0%
Terminal rate	3/33 (9%)	0/42 (0%)	0/33 (0%)	0/23 (0%)
First incidence (days)	728 (T)	—	—	—
Poly-3 test	P=0.138N	P=0.115N	P=0.117N	P=0.148N
Skin: Fibrosarcoma or Sarcoma				
Overall rate	3/50 (6%)	0/50 (0%)	1/50 (2%)	1/50 (2%)
Adjusted rate	6.6%	0.0%	2.2%	2.5%
Terminal rate	3/33 (9%)	0/42 (0%)	0/33 (0%)	0/23 (0%)
First incidence (days)	728 (T)	—	659	583
Poly-3 test	P=0.480N	P=0.115N	P=0.300N	P=0.357N
Skin: Fibroma, Fibrosarcoma, or Sarcoma				
Overall rate	4/50 (8%)	4/50 (8%)	2/50 (4%)	1/50 (2%)
Adjusted rate	8.7%	8.5%	4.3%	2.5%
Terminal rate	4/33 (12%)	3/42 (7%)	1/33 (3%)	0/23 (0%)
First incidence (days)	728 (T)	611	659	583
Poly-3 test	P=0.145N	P=0.627N	P=0.331N	P=0.224N
Thyroid Gland (C-cell): Adenoma				
Overall rate	9/49 (18%)	3/47 (6%)	5/47 (11%)	2/45 (4%)
Adjusted rate	19.6%	6.7%	11.3%	5.4%
Terminal rate	6/33 (18%)	3/42 (7%)	3/33 (9%)	0/23 (0%)
First incidence (days)	547	728 (T)	659	701
Poly-3 test	P=0.109N	P=0.065N	P=0.215N	P=0.057N

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	9/49 (18%)	3/47 (6%)	6/47 (13%)	2/45 (4%)
Adjusted rate	19.6%	6.7%	13.5%	5.4%
Terminal rate	6/33 (18%)	3/42 (7%)	3/33 (9%)	0/23 (0%)
First incidence (days)	547	728 (T)	646	701
Poly-3 test	P=0.117N	P=0.065N	P=0.311N	P=0.057N
Uterus: Stromal Polyp				
Overall rate	3/50 (6%)	9/50 (18%)	4/50 (8%)	8/50 (16%)
Adjusted rate	6.5%	19.3%	8.7%	20.1%
Terminal rate	2/33 (6%)	9/42 (21%)	3/33 (9%)	4/23 (17%)
First incidence (days)	673	728 (T)	714	694
Poly-3 test	P=0.150	P=0.063	P=0.503	P=0.058
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	3/50 (6%)	9/50 (18%)	5/50 (10%)	8/50 (16%)
Adjusted rate	6.5%	19.3%	10.8%	20.1%
Terminal rate	2/33 (6%)	9/42 (21%)	4/33 (12%)	4/23 (17%)
First incidence (days)	673	728 (T)	714	694
Poly-3 test	P=0.151	P=0.063	P=0.359	P=0.058
All Organs: Mononuclear Cell Leukemia				
Overall rate	15/50 (30%)	2/50 (4%)	1/50 (2%)	1/50 (2%)
Adjusted rate	31.7%	4.2%	2.2%	2.5%
Terminal rate	8/33 (24%)	1/42 (2%)	0/33 (0%)	1/23 (4%)
First incidence (days)	547	516	705	728 (T)
Poly-3 test	P<0.001N	P<0.001N	P<0.001N	P<0.001N
All Organs: Benign Neoplasms				
Overall rate	46/50 (92%)	44/50 (88%)	42/50 (84%)	33/50 (66%)
Adjusted rate	92.0%	91.7%	86.0%	77.1%
Terminal rate	29/33 (88%)	39/42 (93%)	28/33 (85%)	18/23 (78%)
First incidence (days)	547	574	604	471
Poly-3 test	P=0.011N	P=0.628N	P=0.258N	P=0.032N
All Organs: Malignant Neoplasms				
Overall rate	23/50 (46%)	9/50 (18%)	12/50 (24%)	11/50 (22%)
Adjusted rate	47.8%	18.8%	25.2%	26.6%
Terminal rate	14/33 (42%)	7/42 (17%)	6/33 (18%)	6/23 (26%)
First incidence (days)	547	516	611	468
Poly-3 test	P=0.156N	P=0.002N	P=0.016N	P=0.030N

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	49/50 (98%)	46/50 (92%)	43/50 (86%)	36/50 (72%)
Adjusted rate	98.0%	94.6%	87.3%	81.3%
Terminal rate	32/33 (97%)	40/42 (95%)	28/33 (85%)	18/23 (78%)
First incidence (days)	547	516	604	468
Poly-3 test	P=0.002N	P=0.358N	P=0.042N	P=0.004N

(T) Terminal kill
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal cortex, clitoral gland, liver, ovary, pituitary gland, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in a dose group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

TABLE B3a
Historical Incidence of Hepatocellular Neoplasms in Control Female F344/N Rats[a]

Study (Study Start)	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Corn Oil Gavage Studies			
N,N-Dimethyl-*p*-toluidine (October 2004)	0/50	0/50	0/50
Ginkgo biloba extract (March 2005)	0/50	0/50	0/50
Isoeugenol (April 2002)	0/50	0/50	0/50
Kava kava extract (August 2004)	0/50	0/50	0/50
β-Myrcene (March 2002)	0/50	0/50	0/50
Pulegone (April 2003)	1/50	0/50	1/50
Total (%)	1/300 (0.3%)	0/300	1/300 (0.3%)
Mean ± standard deviation	0.3% ± 0.8%		0.3% ± 0.8%
Range	0%-2%		0%-2%
Overall Historical Incidence: All Routes			
Total (%)	11/1,200 (0.9%)	1/1,200 (0.1%)	12/1,200 (1.0%)
Mean ± standard deviation	0.9% ± 1.6%	0.1% ± 0.4%	1.0% ± 1.6%
Range	0%-4%	0%-2%	0%-4%

[a] Data as of May 2011

TABLE B3b
Historical Incidence of Adenoma of the Nose in Control Female F344/N Rats[a]

Study (Study Start)	Incidence in Controls
Historical Incidence: Corn Oil Gavage Studies	
N,N-Dimethyl-*p*-toluidine (October 2004)	0/50
Ginkgo biloba extract (March 2005)	0/49
Isoeugenol (April 2002)	0/50
Kava kava extract (August 2004)	0/50
β-Myrcene (March 2002)	0/50
Pulegone (April 2003)	0/50
Total	0/299
Overall Historical Incidence: All Routes	
Total (%)	1/1,196 (0.1%)
Mean ± standard deviation	0.1% ± 0.4%
Range	0%-2%

[a] Data as of May 2011

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine[a]

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths		1		1
Moribund	14	3	8	8
Natural deaths	3	4	9	18
Survivors				
Terminal kill	33	42	33	23
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Parasite metazoan		1 (2%)		
Intestine large, rectum	(50)	(50)	(50)	(50)
Parasite metazoan	4 (8%)	4 (8%)	3 (6%)	3 (6%)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Hyperplasia, lymphoid		1 (2%)		
Liver	(50)	(50)	(50)	(49)
Angiectasis	1 (2%)	4 (8%)	10 (20%)	5 (10%)
Basophilic focus	46 (92%)	45 (90%)	5 (10%)	6 (12%)
Clear cell focus	7 (14%)	17 (34%)	24 (48%)	29 (59%)
Degeneration, cystic			2 (4%)	10 (20%)
Eosinophilic focus	18 (36%)	24 (48%)	29 (58%)	32 (65%)
Fatty change, focal	8 (16%)	13 (26%)	3 (6%)	4 (8%)
Fatty change, diffuse	9 (18%)	1 (2%)	3 (6%)	1 (2%)
Hematopoietic cell proliferation			2 (4%)	2 (4%)
Hepatodiaphragmatic nodule	3 (6%)	6 (12%)	5 (10%)	3 (6%)
Inflammation	38 (76%)	46 (92%)	42 (84%)	39 (80%)
Mixed cell focus	14 (28%)	20 (40%)	17 (34%)	26 (53%)
Bile duct, cyst		1 (2%)		1 (2%)
Bile duct, fibrosis	6 (12%)	11 (22%)	23 (46%)	27 (55%)
Bile duct, hyperplasia	10 (20%)	21 (42%)	27 (54%)	43 (88%)
Centrilobular, degeneration			1 (2%)	1 (2%)
Hepatocyte, hypertrophy			6 (12%)	22 (45%)
Hepatocyte, necrosis			1 (2%)	5 (10%)
Oval cell, hyperplasia	2 (4%)		2 (4%)	1 (2%)
Mesentery	(8)	(9)	(9)	(3)
Fat, necrosis	8 (100%)	9 (100%)	9 (100%)	3 (100%)
Pancreas	(50)	(50)	(50)	(50)
Cyst	5 (10%)	4 (8%)	5 (10%)	5 (10%)
Fibrosis	1 (2%)			
Infiltration cellular, mononuclear cell	13 (26%)	11 (22%)	9 (18%)	9 (18%)
Acinus, atrophy	14 (28%)	7 (14%)	9 (18%)	7 (14%)
Acinus, hyperplasia				1 (2%)
Duct, fibrosis	1 (2%)			
Duct, inflammation, chronic active		1 (2%)		
Salivary glands	(50)	(50)	(50)	(48)
Stomach, forestomach	(50)	(50)	(50)	(50)
Hyperplasia, squamous	5 (10%)	1 (2%)	4 (8%)	4 (8%)
Inflammation	5 (10%)		4 (8%)	2 (4%)
Ulcer	5 (10%)		3 (6%)	2 (4%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Alimentary System (continued)				
Stomach, glandular	(50)	(50)	(50)	(50)
Inflammation			1 (2%)	
Mineralization	1 (2%)			
Ulcer			1 (2%)	
Tongue	(1)	(0)	(0)	(2)
Tooth	(0)	(1)	(0)	(0)
Peridontal tissue, inflammation		1 (100%)		
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	36 (72%)	42 (84%)	40 (80%)	42 (84%)
Pigmentation			1 (2%)	
Thrombosis				1 (2%)
Endocardium, hyperplasia		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(49)
Angiectasis	39 (78%)	42 (84%)	43 (86%)	34 (69%)
Degeneration, cystic	8 (16%)	5 (10%)	5 (10%)	3 (6%)
Hyperplasia	26 (52%)	24 (48%)	28 (56%)	12 (24%)
Hypertrophy	11 (22%)	10 (20%)	12 (24%)	8 (16%)
Necrosis	1 (2%)			1 (2%)
Pigmentation		1 (2%)		
Vacuolization cytoplasmic	26 (52%)	26 (52%)	26 (52%)	18 (37%)
Adrenal medulla	(50)	(50)	(50)	(49)
Atrophy		1 (2%)		
Hyperplasia	4 (8%)	3 (6%)	1 (2%)	5 (10%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Parathyroid gland	(50)	(50)	(50)	(46)
Hyperplasia, focal			1 (2%)	
Hyperplasia, diffuse	1 (2%)	2 (4%)	2 (4%)	5 (11%)
Pituitary gland	(50)	(50)	(50)	(50)
Cyst	11 (22%)	20 (40%)	15 (30%)	4 (8%)
Fibrosis		1 (2%)		
Pigmentation		1 (2%)		
Pars distalis, angiectasis	1 (2%)			1 (2%)
Pars distalis, cyst	4 (8%)		2 (4%)	1 (2%)
Pars distalis, hyperplasia	14 (28%)	17 (34%)	15 (30%)	17 (34%)
Thyroid gland	(49)	(47)	(47)	(45)
C-cell, hyperplasia	29 (59%)	33 (70%)	15 (32%)	4 (9%)
Follicle, cyst	1 (2%)			
Follicular cell, hyperplasia	1 (2%)	1 (2%)		
General Body System				
None				

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Genital System				
Clitoral gland	(50)	(49)	(50)	(50)
Cyst	11 (22%)	8 (16%)	7 (14%)	5 (10%)
Hyperplasia	9 (18%)	9 (18%)	6 (12%)	4 (8%)
Inflammation	22 (44%)	26 (53%)	20 (40%)	15 (30%)
Ovary	(50)	(50)	(50)	(50)
Atrophy		2 (4%)		
Cyst	4 (8%)	3 (6%)	5 (10%)	
Uterus	(50)	(50)	(50)	(50)
Cyst	1 (2%)			
Decidual reaction			1 (2%)	
Hemorrhage				2 (4%)
Inflammation	1 (2%)		1 (2%)	
Cervix, cyst				1 (2%)
Endometrium, hyperplasia, cystic				2 (4%)
Myometrium, fibrosis		1 (2%)		
Vagina	(1)	(1)	(0)	(0)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	18 (36%)	13 (26%)	18 (36%)	49 (98%)
Myelofibrosis	1 (2%)			1 (2%)
Lymph node	(2)	(1)	(2)	(1)
Mediastinal, ectasia	1 (50%)	1 (100%)	1 (50%)	1 (100%)
Lymph node, mesenteric	(50)	(49)	(50)	(49)
Hyperplasia, lymphoid	1 (2%)			
Infiltration cellular, histiocyte	30 (60%)	29 (59%)	35 (70%)	33 (67%)
Spleen	(50)	(50)	(50)	(50)
Congestion		9 (18%)	26 (52%)	28 (56%)
Hematopoietic cell proliferation	32 (64%)	45 (90%)	47 (94%)	42 (84%)
Necrosis	2 (4%)			
Pigmentation	44 (88%)	47 (94%)	47 (94%)	49 (98%)
Capsule, fibrosis	8 (16%)		8 (16%)	41 (82%)
Capsule, hemorrhage		1 (2%)		
Capsule, hypertrophy, mesothelium	1 (2%)	14 (28%)	10 (20%)	16 (32%)
Lymphoid follicle, atrophy	1 (2%)	2 (4%)		28 (56%)
Red pulp, hyperplasia		1 (2%)		
Thymus	(47)	(50)	(50)	(48)
Atrophy	45 (96%)	45 (90%)	45 (90%)	44 (92%)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Cyst	4 (8%)		3 (6%)	
Hyperplasia	9 (18%)	9 (18%)	2 (4%)	
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion		1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hyperostosis			1 (2%)	

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hemorrhage		1 (2%)	1 (2%)	
Hydrocephalus		1 (2%)		
Peripheral nerve	(0)	(0)	(1)	(0)
Spinal cord	(0)	(0)	(1)	(0)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion				2 (4%)
Fibrosis	1 (2%)			
Inflammation	3 (6%)			1 (2%)
Metaplasia, squamous	2 (4%)			
Alveolar epithelium, hyperplasia	5 (10%)	2 (4%)	2 (4%)	2 (4%)
Alveolus, infiltration cellular, histiocyte	11 (22%)	10 (20%)	11 (22%)	17 (34%)
Nose	(50)	(49)	(50)	(49)
Foreign body	3 (6%)	8 (16%)	1 (2%)	4 (8%)
Inflammation	23 (46%)	24 (49%)	22 (44%)	45 (92%)
Glands, hyperplasia				1 (2%)
Glands, olfactory epithelium, dilatation				48 (98%)
Glands, olfactory epithelium, hyperplasia			4 (8%)	47 (96%)
Glands, olfactory epithelium, metaplasia				42 (86%)
Glands, olfactory epithelium, necrosis				18 (37%)
Glands, respiratory epithelium, dilatation	5 (10%)	12 (24%)	27 (54%)	47 (96%)
Glands, respiratory epithelium, hyperplasia	6 (12%)	9 (18%)	22 (44%)	45 (92%)
Glands, respiratory epithelium, metaplasia, respiratory	17 (34%)	33 (67%)	44 (88%)	47 (96%)
Glands, transitional epithelium, dilatation				9 (18%)
Glands, transitional epithelium, hyperplasia		4 (8%)	12 (24%)	24 (49%)
Nasolacrimal duct, inflammation	1 (2%)			
Nerve, atrophy				4 (8%)
Olfactory epithelium, accumulation, hyaline droplet	43 (86%)	42 (86%)	38 (76%)	
Olfactory epithelium, degeneration			1 (2%)	46 (94%)
Olfactory epithelium, hyperplasia, basal cell				25 (51%)
Olfactory epithelium, metaplasia, respiratory	4 (8%)	6 (12%)	1 (2%)	21 (43%)
Olfactory epithelium, metaplasia, squamous				2 (4%)
Respiratory epithelium, accumulation, hyaline droplet	35 (70%)	30 (61%)	23 (46%)	2 (4%)
Respiratory epithelium, hyperplasia	10 (20%)	13 (27%)	11 (22%)	41 (84%)
Transitional epithelium, degeneration				1 (2%)
Transitional epithelium, hyperplasia		1 (2%)	6 (12%)	33 (67%)
Trachea	(50)	(50)	(50)	(50)
Inflammation				1 (2%)
Inflammation, suppurative				1 (2%)
Perforation				1 (2%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Special Senses System				
Ear	(1)	(0)	(0)	(1)
Eye	(50)	(50)	(50)	(50)
Cataract	3 (6%)	2 (4%)	3 (6%)	
Ciliary body, cornea, inflammation	1 (2%)			
Cornea, degeneration				1 (2%)
Retina, atrophy	2 (4%)	3 (6%)	3 (6%)	1 (2%)
Harderian gland	(50)	(50)	(50)	(50)
Inflammation	6 (12%)	4 (8%)	2 (4%)	1 (2%)
Lacrimal gland	(0)	(0)	(0)	(1)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet	25 (50%)	23 (46%)	5 (10%)	1 (2%)
Calculus microscopic observation only			1 (2%)	
Cyst		1 (2%)		1 (2%)
Infarct	4 (8%)		5 (10%)	2 (4%)
Mineralization	33 (66%)	35 (70%)	35 (70%)	37 (74%)
Nephropathy	28 (56%)	38 (76%)	38 (76%)	41 (82%)
Pigmentation	41 (82%)	45 (90%)	43 (86%)	49 (98%)
Papilla, fibrosis	1 (2%)		1 (2%)	
Papilla, inflammation	1 (2%)			
Papilla, necrosis				1 (2%)
Pelvis, dilatation				1 (2%)
Pelvis, inflammation	3 (6%)	3 (6%)	9 (18%)	5 (10%)
Pelvis, transitional epithelium, hyperplasia	2 (4%)	3 (6%)	8 (16%)	6 (12%)
Renal tubule, dilatation	1 (2%)			
Renal tubule, hyperplasia	1 (2%)			
Urinary bladder	(50)	(50)	(50)	(50)
Inflammation				1 (2%)

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR GAVAGE STUDY OF *N*,*N*-DIMETHYL-*p*-TOLUIDINE

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine[a]

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	2			
Moribund	5	4	11	14
Natural deaths	9	10	8	
Survivors				
Died last week of study	1			
Terminal kill	33	36	31	36
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(49)	(50)	(47)	(49)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Adenoma		1 (2%)		1 (2%)
Carcinoma, multiple				1 (2%)
Liver	(50)	(50)	(50)	(50)
Carcinoma, metastatic, islets, pancreatic	1 (2%)			
Fibrous histiocytoma, metastatic, skin		1 (2%)		
Hemangioma			1 (2%)	
Hemangiosarcoma	6 (12%)	2 (4%)	9 (18%)	3 (6%)
Hepatoblastoma	1 (2%)	5 (10%)	8 (16%)	8 (16%)
Hepatoblastoma, multiple			2 (4%)	
Hepatocellular adenoma	12 (24%)	15 (30%)	10 (20%)	10 (20%)
Hepatocellular adenoma, multiple	17 (34%)	19 (38%)	27 (54%)	26 (52%)
Hepatocellular carcinoma	15 (30%)	18 (36%)	14 (28%)	14 (28%)
Hepatocellular carcinoma, multiple	7 (14%)	7 (14%)	16 (32%)	22 (44%)
Hepatocholangiocarcinoma			1 (2%)	1 (2%)
Mesentery	(4)	(3)	(5)	(2)
Fat, hepatocholangiocarcinoma, metastatic, liver			1 (20%)	
Pancreas	(50)	(50)	(50)	(50)
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Salivary glands	(50)	(50)	(50)	(50)
Hepatocellular carcinoma, metastatic, liver		1 (2%)		
Stomach, forestomach	(50)	(50)	(50)	(50)
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Squamous cell papilloma	1 (2%)	1 (2%)		3 (6%)
Stomach, glandular	(50)	(50)	(50)	(50)
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Tongue	(2)	(0)	(0)	(0)
Tooth	(37)	(38)	(34)	(30)
Odontoma	3 (8%)	1 (3%)	1 (3%)	
Odontoma, multiple	2 (5%)			

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Carcinoma, metastatic, Zymbal's gland	1 (2%)			
Hemangiosarcoma	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma				1 (2%)
Bilateral, subscapular, adenoma		1 (2%)		
Subscapular, adenoma	3 (6%)	4 (8%)	1 (2%)	1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign				1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	1 (2%)		
Adenoma, multiple		1 (2%)		
Carcinoma	1 (2%)			
Parathyroid gland	(40)	(43)	(45)	(42)
Pituitary gland	(50)	(50)	(49)	(50)
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, adenoma	1 (2%)			
General Body System				
None				
Genital System				
Coagulating gland	(0)	(1)	(0)	(0)
Epididymis	(50)	(50)	(50)	(50)
Hemangiosarcoma			1 (2%)	
Preputial gland	(50)	(50)	(50)	(50)
Prostate	(50)	(50)	(50)	(50)
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma				1 (2%)
Lymph node	(2)	(3)	(4)	(0)
Mediastinal, hepatocholangiocarcinoma, metastatic, liver			1 (25%)	
Pancreatic, hemangiosarcoma			1 (25%)	
Lymph node, mandibular	(50)	(50)	(49)	(50)
Lymph node, mesenteric	(50)	(50)	(49)	(50)
Hemangiosarcoma	1 (2%)			
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Spleen	(48)	(50)	(49)	(50)
Hemangiosarcoma	3 (6%)	1 (2%)	3 (6%)	1 (2%)
Hemangiosarcoma, multiple			1 (2%)	
Thymus	(48)	(48)	(48)	(49)
Carcinoma, metastatic, Zymbal's gland	1 (2%)			

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Subcutaneous tissue, fibrosarcoma				1 (2%)
Subcutaneous tissue, fibrous histiocytoma		1 (2%)		
Subcutaneous tissue, lipoma	1 (2%)	1 (2%)		
Subcutaneous tissue, melanoma malignant		1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(0)	(1)	(2)	(2)
Hemangiosarcoma			1 (50%)	
Hepatocholangiocarcinoma, metastatic, liver			1 (50%)	
Sarcoma				1 (50%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	11 (22%)	11 (22%)	17 (34%)	10 (20%)
Alveolar/bronchiolar adenoma, multiple		5 (10%)	1 (2%)	
Alveolar/bronchiolar carcinoma	2 (4%)	2 (4%)		4 (8%)
Alveolar/bronchiolar carcinoma, multiple		1 (2%)		
Carcinoma, metastatic, harderian gland			1 (2%)	
Carcinoma, metastatic, Zymbal's gland	1 (2%)			
Hemangiosarcoma	1 (2%)			
Hepatoblastoma, metastatic, liver		2 (4%)	4 (8%)	5 (10%)
Hepatocellular carcinoma, metastatic, liver	6 (12%)	5 (10%)	7 (14%)	3 (6%)
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Osteosarcoma, metastatic, uncertain primary site		1 (2%)		
Bronchus, adenoma		1 (2%)		
Nose	(49)	(50)	(50)	(50)
Respiratory epithelium, adenoma		1 (2%)		
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Ear	(1)	(0)	(0)	(0)
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	10 (20%)	7 (14%)	4 (8%)	2 (4%)
Carcinoma		1 (2%)	1 (2%)	
Bilateral, adenoma	1 (2%)			
Zymbal's gland	(1)	(0)	(0)	(0)
Carcinoma	1 (100%)			

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Hepatocellular carcinoma, metastatic, liver		1 (2%)		
Renal tubule, adenoma	1 (2%)			
Ureter	(1)	(0)	(0)	(0)
Urethra	(0)	(1)	(0)	(0)
Urinary bladder	(50)	(50)	(50)	(50)
Hemangioma				1 (2%)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)	
Lymphoma malignant	4 (8%)	6 (12%)	1 (2%)	1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	45	47	50	49
Total primary neoplasms	107	115	122	114
Total animals with benign neoplasms	36	40	41	39
Total benign neoplasms	64	70	62	56
Total animals with malignant neoplasms	29	32	41	40
Total malignant neoplasms	43	45	60	58
Total animals with metastatic neoplasms	8	9	12	8
Total metastatic neoplasms	10	11	21	8
Total animals with malignant neoplasms of uncertain primary site		1		

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Adrenal Cortex: Adenoma				
Overall rate[a]	3/50 (6%)	5/50 (10%)	1/50 (2%)	2/50 (4%)
Adjusted rate[b]	6.9%	11.1%	2.3%	4.5%
Terminal rate[c]	1/34 (3%)	5/36 (14%)	1/31 (3%)	2/36 (6%)
First incidence (days)	561	730 (T)	730 (T)	730 (T)
Poly-3 test[d]	P=0.263N	P=0.374	P=0.314N	P=0.492N
Harderian Gland: Adenoma				
Overall rate	11/50 (22%)	7/50 (14%)	4/50 (8%)	2/50 (4%)
Adjusted rate	25.3%	15.2%	9.2%	4.5%
Terminal rate	8/34 (24%)	5/36 (14%)	3/31 (10%)	2/36 (6%)
First incidence (days)	584	599	402	730 (T)
Poly-3 test	P=0.008N	P=0.178N	P=0.042N	P=0.006N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	11/50 (22%)	8/50 (16%)	5/50 (10%)	2/50 (4%)
Adjusted rate	25.3%	17.4%	11.3%	4.5%
Terminal rate	8/34 (24%)	5/36 (14%)	3/31 (10%)	2/36 (6%)
First incidence (days)	584	599	402	730 (T)
Poly-3 test	P=0.006N	P=0.255N	P=0.075N	P=0.006N
Liver: Hemangiosarcoma				
Overall rate	6/50 (12%)	2/50 (4%)	9/50 (18%)	3/50 (6%)
Adjusted rate	14.0%	4.4%	20.6%	6.6%
Terminal rate	4/34 (12%)	1/36 (3%)	5/31 (16%)	1/36 (3%)
First incidence (days)	678	694	597	623
Poly-3 test	P=0.333N	P=0.115N	P=0.296	P=0.217N
Liver: Hepatocellular Adenoma				
Overall rate	29/50 (58%)	34/50 (68%)	37/50 (74%)	36/50 (72%)
Adjusted rate	65.7%	72.5%	81.0%	76.3%
Terminal rate	23/34 (68%)	28/36 (78%)	28/31 (90%)	28/36 (78%)
First incidence (days)	561	550	548	449
Poly-3 test	P=0.219	P=0.312	P=0.069	P=0.180
Liver: Hepatocellular Carcinoma				
Overall rate	22/50 (44%)	25/50 (50%)	30/50 (60%)	36/50 (72%)
Adjusted rate	48.9%	52.2%	65.1%	75.7%
Terminal rate	16/34 (47%)	17/36 (47%)	19/31 (61%)	27/36 (75%)
First incidence (days)	548	539	442	562
Poly-3 test	P=0.002	P=0.458	P=0.084	P=0.005
Liver: Hepatocellular Adenoma or Hepatocellular Carcinoma				
Overall rate	38/50 (76%)	44/50 (88%)	47/50 (94%)	48/50 (96%)
Adjusted rate	83.1%	90.6%	98.0%	98.6%
Terminal rate	28/34 (82%)	34/36 (94%)	31/31 (100%)	36/36 (100%)
First incidence (days)	548	539	442	449
Poly-3 test	P=0.005	P=0.206	P=0.010	P=0.006
Liver: Hepatoblastoma				
Overall rate	1/50 (2%)	5/50 (10%)	10/50 (20%)	8/50 (16%)
Adjusted rate	2.3%	10.8%	22.3%	17.3%
Terminal rate	1/34 (3%)	3/36 (8%)	4/31 (13%)	3/36 (8%)
First incidence (days)	730 (T)	539	512	580
Poly-3 test	P=0.064	P=0.121	P=0.005	P=0.021

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	22/50 (44%)	28/50 (56%)	34/50 (68%)	37/50 (74%)
Adjusted rate	48.9%	57.8%	72.2%	77.6%
Terminal rate	16/34 (47%)	19/36 (53%)	21/31 (68%)	27/36 (75%)
First incidence (days)	548	539	442	562
Poly-3 test	P=0.003	P=0.256	P=0.016	P=0.003
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	38/50 (76%)	45/50 (90%)	48/50 (96%)	48/50 (96%)
Adjusted rate	83.1%	91.7%	99.2%	98.6%
Terminal rate	28/34 (82%)	34/36 (94%)	31/31 (100%)	36/36 (100%)
First incidence (days)	548	539	442	449
Poly-3 test	P=0.006	P=0.157	P=0.004	P=0.006
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	11/50 (22%)	16/50 (32%)	18/50 (36%)	10/50 (20%)
Adjusted rate	25.4%	34.7%	41.1%	22.2%
Terminal rate	9/34 (27%)	13/36 (36%)	13/31 (42%)	8/36 (22%)
First incidence (days)	643	568	609	609
Poly-3 test	P=0.204N	P=0.233	P=0.089	P=0.457N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	2/50 (4%)	3/50 (6%)	0/50 (0%)	4/50 (8%)
Adjusted rate	4.7%	6.6%	0.0%	8.8%
Terminal rate	2/34 (6%)	3/36 (8%)	0/31 (0%)	2/36 (6%)
First incidence (days)	730 (T)	730 (T)	—[e]	562
Poly-3 test	P=0.271	P=0.526	P=0.237N	P=0.364
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	13/50 (26%)	19/50 (38%)	18/50 (36%)	12/50 (24%)
Adjusted rate	30.0%	41.2%	41.1%	26.2%
Terminal rate	11/34 (32%)	16/36 (44%)	13/31 (42%)	8/36 (22%)
First incidence (days)	643	568	609	562
Poly-3 test	P=0.167N	P=0.187	P=0.194	P=0.433N
Spleen: Hemangiosarcoma				
Overall rate	3/48 (6%)	1/50 (2%)	4/49 (8%)	1/50 (2%)
Adjusted rate	7.3%	2.2%	9.2%	2.2%
Terminal rate	3/34 (9%)	0/36 (0%)	2/31 (7%)	1/36 (3%)
First incidence (days)	730 (T)	697	548	730 (T)
Poly-3 test	P=0.332N	P=0.272N	P=0.526	P=0.278N
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	1/50 (2%)	1/50 (2%)	0/50 (0%)	3/50 (6%)
Adjusted rate	2.3%	2.2%	0.0%	6.7%
Terminal rate	1/34 (3%)	1/36 (3%)	0/31 (0%)	2/36 (6%)
First incidence (days)	730 (T)	730 (T)	—	665
Poly-3 test	P=0.132	P=0.748N	P=0.500N	P=0.322
Tooth: Odontoma				
Overall rate	5/50 (10%)	1/50 (2%)	1/50 (2%)	0/50 (0%)
Adjusted rate	11.5%	2.2%	2.3%	0.0%
Terminal rate	4/34 (12%)	1/36 (3%)	0/31 (0%)	0/36 (0%)
First incidence (days)	561	730 (T)	710	—
Poly-3 test	P=0.042N	P=0.091N	P=0.104N	P=0.028N

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
All Organs: Hemangiosarcoma				
Overall rate	8/50 (16%)	3/50 (6%)	12/50 (24%)	4/50 (8%)
Adjusted rate	18.6%	6.6%	27.0%	8.9%
Terminal rate	6/34 (18%)	1/36 (3%)	6/31 (19%)	2/36 (6%)
First incidence (days)	678	694	548	623
Poly-3 test	P=0.269N	P=0.081N	P=0.247	P=0.152N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	8/50 (16%)	3/50 (6%)	13/50 (26%)	5/50 (10%)
Adjusted rate	18.6%	6.6%	29.3%	11.1%
Terminal rate	6/34 (18%)	1/36 (3%)	7/31 (23%)	3/36 (8%)
First incidence (days)	678	694	548	623
Poly-3 test	P=0.391N	P=0.081N	P=0.178	P=0.243N
All Organs: Malignant Lymphoma				
Overall rate	4/50 (8%)	6/50 (12%)	1/50 (2%)	1/50 (2%)
Adjusted rate	9.2%	13.2%	2.3%	2.2%
Terminal rate	2/34 (6%)	5/36 (14%)	0/31 (0%)	1/36 (3%)
First incidence (days)	584	714	597	730 (T)
Poly-3 test	P=0.055N	P=0.398	P=0.179N	P=0.169N
All Organs: Benign Neoplasms				
Overall rate	36/50 (72%)	40/50 (80%)	41/50 (82%)	39/50 (78%)
Adjusted rate	79.2%	83.8%	87.0%	82.6%
Terminal rate	27/34 (79%)	31/36 (86%)	29/31 (94%)	31/36 (86%)
First incidence (days)	561	550	402	449
Poly-3 test	P=0.503	P=0.375	P=0.225	P=0.438
All Organs: Malignant Neoplasms				
Overall rate	29/50 (58%)	32/50 (64%)	41/50 (82%)	40/50 (80%)
Adjusted rate	63.5%	65.0%	84.3%	82.9%
Terminal rate	20/34 (59%)	20/36 (56%)	25/31 (81%)	28/36 (78%)
First incidence (days)	548	539	442	562
Poly-3 test	P=0.013	P=0.524	P=0.016	P=0.026
All Organs: Benign or Malignant Neoplasms				
Overall rate	45/50 (90%)	47/50 (94%)	50/50 (100%)	49/50 (98%)
Adjusted rate	95.7%	95.1%	100.0%	99.9%
Terminal rate	32/34 (94%)	34/36 (94%)	31/31 (100%)	36/36 (100%)
First incidence (days)	548	539	402	449
Poly-3 test	P=0.102	P=0.632N	P=0.222	P=0.235

(T) Terminal kill

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, lung, and spleen; for other tissues, denominator is number of animals necropsied.

[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in a dose group is indicated by **N**.

[e] Not applicable; no neoplasms in animal group

TABLE C3a
Historical Incidence of Liver Neoplasms in Control Male B6C3F1/N Mice[a]

Study (Study Start)	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Hepatocellular Carcinoma
Historical Incidence: Corn Oil Gavage Studies			
N,*N*-Dimethyl-*p*-toluidine (October 2004)	29/50	22/50	38/50
Ginkgo biloba extract (March 2005)	31/50	22/50	39/50
Isoeugenol (May 2002)	24/50	8/50	28/50
Kava kava extract (August 2004)	27/50	20/50	38/50
β-Myrcene (April 2002)	26/50	14/50	33/50
Pulegone (April 2003)	22/50	13/50	29/50
3,3′,4,4′-Tetrachloroazobenzene (February 2003)	22/50	17/50	34/50
Total (%)	181/350 (51.7%)	116/350 (33.1%)	239/350 (68.3%)
Mean ± standard deviation	51.7% ± 6.9%	33.1% ± 10.5%	68.3% ± 8.9%
Range	44%-62%	16%-44%	56%-78%
Overall Historical Incidence: All Routes			
Total (%)	658/1,149 (57.3%)	399/1,149 (34.7%)	844/1,149 (73.5%)
Mean ± standard deviation	57.3% ± 12.6%	34.7% ± 10.8%	73.5% ± 11.3%
Range	24%-78%	16%-56%	52%-90%

	Hepatoblastoma	Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma
Historical Incidence: Corn Oil Gavage Studies		
N,*N*-Dimethyl-*p*-toluidine (October 2004)	1/50	38/50
Ginkgo biloba extract (March 2005)	3/50	39/50
Isoeugenol (May 2002)	3/50	30/50
Kava kava extract (August 2004)	0/50	38/50
β-Myrcene (April 2002)	4/50	34/50
Pulegone (April 2003)	1/50	29/50
3,3′,4,4′-Tetrachloroazobenzene (February 2003)	2/50	34/50
Total (%)	14/350 (4.0%)	242/350 (69.1%)
Mean ± standard deviation	4.0% ± 2.8%	69.1% ± 8.0%
Range	0%-8%	58%-78%
Overall Historical Incidence: All Routes		
Total (%)	61/1,149 (5.3%)	852/1,149 (74.2%)
Mean ± standard deviation	5.3% ± 7.1%	74.2% ± 11.5%
Range	0%-34%	52%-92%

[a] Data as of May 2011

TABLE C3b
Historical Incidence of Alveolar/bronchiolar Neoplasms in Control Male B6C3F1/N Mice[a]

Study (Study Start)	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Corn Oil Gavage Studies			
N,*N*-Dimethyl-*p*-toluidine (October 2004)	11/50	2/50	13/50
Ginkgo biloba extract (March 2005)	8/50	11/50	17/50
Isoeugenol (May 2002)	6/50	2/50	7/50
Kava kava extract (August 2004)	9/50	2/50	11/50
β-Myrcene (April 2002)	8/50	5/50	13/50
Pulegone (April 2003)	6/50	3/50	9/50
3,3′,4,4′-Tetrachloroazobenzene (February 2003)	5/50	3/50	7/50
Total (%)	53/350 (15.1%)	28/350 (8.0%)	77/350 (22.0%)
Mean ± standard deviation	15.1% ± 4.1%	8.0% ± 6.5%	22.0% ± 7.3%
Range	10%-22%	4%-22%	14%-34%
Overall Historical Incidence: All Routes			
Total (%)	172/1,150 (15.0%)	144/1,150 (12.5%)	301/1,150 (26.2%)
Mean ± standard deviation	15.0% ± 6.9%	12.5% ± 7.1%	26.2% ± 6.3%
Range	2%-30%	4%-24%	14%-40%

[a] Data as of May 2011

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine[a]

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	2			
Moribund	5	4	11	14
Natural deaths	9	10	8	
Survivors				
Died last week of study	1			
Terminal kill	33	36	31	36
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Necrosis	1 (2%)			
Perforation	1 (2%)			
Periesophageal tissue, inflammation	2 (4%)			
Gallbladder	(49)	(50)	(47)	(49)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Peyer's patch, hyperplasia		1 (2%)		
Liver	(50)	(50)	(50)	(50)
Angiectasis	3 (6%)	1 (2%)		
Basophilic focus	5 (10%)	11 (22%)	8 (16%)	2 (4%)
Clear cell focus	15 (30%)	22 (44%)	15 (30%)	7 (14%)
Eosinophilic focus	25 (50%)	30 (60%)	39 (78%)	43 (86%)
Fatty change		3 (6%)	1 (2%)	2 (4%)
Hematopoietic cell proliferation	4 (8%)	1 (2%)	4 (8%)	1 (2%)
Inflammation, chronic active	23 (46%)	22 (44%)	18 (36%)	19 (38%)
Mineralization		1 (2%)	1 (2%)	1 (2%)
Mitotic alteration	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Mixed cell focus	21 (42%)	25 (50%)	17 (34%)	12 (24%)
Necrosis	9 (18%)	8 (16%)	7 (14%)	10 (20%)
Pigmentation		3 (6%)	2 (4%)	2 (4%)
Bile duct, cyst		1 (2%)	1 (2%)	
Bile duct, hyperplasia	1 (2%)	1 (2%)	2 (4%)	
Centrilobular, degeneration	1 (2%)		1 (2%)	
Hepatocyte, hypertrophy	1 (2%)	9 (18%)	11 (22%)	16 (32%)
Hepatocyte, karyomegaly	1 (2%)			
Kupffer cell, hyperplasia				1 (2%)
Oval cell, hyperplasia	1 (2%)		1 (2%)	2 (4%)
Mesentery	(4)	(3)	(5)	(2)
Inflammation, suppurative	1 (25%)			
Fat, necrosis	2 (50%)	3 (100%)	5 (100%)	2 (100%)
Vein, thrombosis	1 (25%)			
Pancreas	(50)	(50)	(50)	(50)
Atrophy		1 (2%)	1 (2%)	1 (2%)
Basophilic focus			1 (2%)	
Inflammation			1 (2%)	
Acinus, hyperplasia				1 (2%)
Duct, cyst			1 (2%)	2 (4%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Alimentary System (continued)				
Salivary glands	(50)	(50)	(50)	(50)
Fibrosis	1 (2%)			
Stomach, forestomach	(50)	(50)	(50)	(50)
Erosion	2 (4%)	1 (2%)		
Hemorrhage				1 (2%)
Inflammation	13 (26%)	12 (24%)	13 (26%)	8 (16%)
Necrosis				1 (2%)
Ulcer	5 (10%)	4 (8%)	5 (10%)	5 (10%)
Epithelium, hyperplasia	14 (28%)	14 (28%)	17 (34%)	11 (22%)
Stomach, glandular	(50)	(50)	(50)	(50)
Inflammation		1 (2%)	1 (2%)	
Mineralization			1 (2%)	
Epithelium, necrosis	1 (2%)	1 (2%)	1 (2%)	
Tongue	(2)	(0)	(0)	(0)
Angiectasis	1 (50%)			
Cyst	1 (50%)			
Tooth	(37)	(38)	(34)	(30)
Dysplasia	34 (92%)	36 (95%)	34 (100%)	26 (87%)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Inflammation			1 (2%)	
Mineralization				1 (2%)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	8 (16%)	7 (14%)	10 (20%)	13 (26%)
Inflammation	2 (4%)			1 (2%)
Mineralization		1 (2%)	2 (4%)	5 (10%)
Atrium, thrombosis		1 (2%)		
Valve, thrombosis	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Hypertrophy	3 (6%)	3 (6%)	1 (2%)	1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)			1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	12 (24%)	5 (10%)	2 (4%)	1 (2%)
Parathyroid gland	(40)	(43)	(45)	(42)
Amyloid deposition			1 (2%)	
Pituitary gland	(50)	(50)	(49)	(50)
Pars distalis, hyperplasia	1 (2%)	1 (2%)		
Thyroid gland	(50)	(50)	(50)	(50)
General Body System				
None				

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Genital System				
Coagulating gland	(0)	(1)	(0)	(0)
Inflammation		1 (100%)		
Epididymis	(50)	(50)	(50)	(50)
Angiectasis				1 (2%)
Granuloma sperm		2 (4%)		
Inflammation				1 (2%)
Preputial gland	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			
Ectasia	7 (14%)	6 (12%)	7 (14%)	8 (16%)
Inflammation		1 (2%)	1 (2%)	1 (2%)
Prostate	(50)	(50)	(50)	(50)
Inflammation	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Epithelium, hyperplasia	1 (2%)			
Seminal vesicle	(50)	(50)	(50)	(50)
Inflammation	1 (2%)	1 (2%)		
Mineralization	1 (2%)			
Testes	(50)	(50)	(50)	(50)
Hyperplasia, oncocytic		1 (2%)		
Germinal epithelium, degeneration		2 (4%)	3 (6%)	
Germinal epithelium, mineralization			1 (2%)	
Interstitial cell, hyperplasia	1 (2%)			1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Atrophy	3 (6%)		1 (2%)	
Hyperplasia	8 (16%)	6 (12%)	9 (18%)	9 (18%)
Necrosis			1 (2%)	
Thrombosis		1 (2%)		
Lymph node	(2)	(3)	(4)	(0)
Lymph node, mandibular	(50)	(50)	(49)	(50)
Atrophy	5 (10%)	4 (8%)	6 (12%)	3 (6%)
Hyperplasia, lymphoid	2 (4%)	1 (2%)	1 (2%)	
Necrosis	1 (2%)			
Lymph node, mesenteric	(50)	(50)	(49)	(50)
Atrophy	13 (26%)	9 (18%)	14 (29%)	15 (30%)
Hyperplasia, lymphoid				2 (4%)
Spleen	(48)	(50)	(49)	(50)
Atrophy	4 (8%)	11 (22%)	11 (22%)	6 (12%)
Hematopoietic cell proliferation	15 (31%)	18 (36%)	23 (47%)	22 (44%)
Hyperplasia, lymphoid	5 (10%)	9 (18%)	6 (12%)	9 (18%)
Necrosis, lymphoid	1 (2%)			
Pigmentation	38 (79%)	34 (68%)	25 (51%)	44 (88%)
Red pulp, atrophy	4 (8%)	1 (2%)	2 (4%)	2 (4%)
Thymus	(48)	(48)	(48)	(49)
Atrophy	41 (85%)	47 (98%)	47 (98%)	48 (98%)
Hyperplasia, lymphoid	1 (2%)			1 (2%)
Infiltration cellular, mast cell				1 (2%)
Necrosis	2 (4%)			
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Inflammation	3 (6%)	1 (2%)	1 (2%)	1 (2%)
Ulcer	4 (8%)	1 (2%)	1 (2%)	2 (4%)
Dermis, fibrosis	2 (4%)			1 (2%)
Epidermis, hyperplasia	1 (2%)		1 (2%)	1 (2%)
Hair follicle, hyperkeratosis				1 (2%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibrosis	1 (2%)			
Fracture		1 (2%)		
Osteopetrosis		1 (2%)		
Skeletal muscle	(0)	(1)	(2)	(2)
Inflammation				1 (50%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hemorrhage			1 (2%)	
Hydrocephalus			1 (2%)	1 (2%)
Necrosis			1 (2%)	
Olfactory lobe, atrophy		1 (2%)		5 (10%)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Foreign body	1 (2%)			
Hemorrhage				1 (2%)
Inflammation, chronic active				1 (2%)
Alveolar epithelium, hyperplasia	3 (6%)	8 (16%)	3 (6%)	
Alveolar epithelium, metaplasia	1 (2%)		1 (2%)	
Alveolus, infiltration cellular, histiocyte	1 (2%)	2 (4%)	2 (4%)	10 (20%)
Artery, inflammation		1 (2%)		
Bronchiole, epithelium, hyperplasia		1 (2%)		
Bronchiole, epithelium, regeneration			1 (2%)	1 (2%)
Bronchus, necrosis			1 (2%)	
Bronchus, epithelium, regeneration				1 (2%)
Mediastinum, inflammation	1 (2%)			
Perivascular, infiltration cellular, lymphoid			1 (2%)	
Serosa, inflammation	1 (2%)			
Nose	(49)	(50)	(50)	(50)
Foreign body		1 (2%)		
Hyperplasia		1 (2%)		
Inflammation	13 (27%)	12 (24%)	10 (20%)	20 (40%)
Polyp, inflammatory	3 (6%)	2 (4%)		
Glands, olfactory epithelium, dilatation	4 (8%)	11 (22%)	7 (14%)	48 (96%)
Glands, olfactory epithelium, hyperplasia	4 (8%)	9 (18%)	7 (14%)	49 (98%)
Glands, olfactory epithelium, metaplasia, respiratory	5 (10%)	5 (10%)	6 (12%)	48 (96%)
Glands, respiratory epithelium, dilatation	17 (35%)	19 (38%)	13 (26%)	41 (82%)
Glands, respiratory epithelium, hyperplasia	4 (8%)	2 (4%)	2 (4%)	11 (22%)
Glands, respiratory epithelium, metaplasia, respiratory	2 (4%)	2 (4%)	2 (4%)	10 (20%)
Nasolacrimal duct, hyperplasia, regenerative				4 (8%)
Nerve, atrophy	2 (4%)	7 (14%)	4 (8%)	42 (84%)
Olfactory epithelium, accumulation, hyaline droplet	12 (24%)	14 (28%)	10 (20%)	4 (8%)
Olfactory epithelium, metaplasia, respiratory	10 (20%)	10 (20%)	5 (10%)	49 (98%)
Olfactory epithelium, necrosis	1 (2%)	3 (6%)	3 (6%)	8 (16%)
Respiratory epithelium, accumulation, hyaline droplet	24 (49%)	25 (50%)	24 (48%)	25 (50%)
Respiratory epithelium, hyperplasia	37 (76%)	35 (70%)	32 (64%)	30 (60%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Respiratory System (continued)				
Nose (continued)	(49)	(50)	(50)	(50)
Respiratory epithelium, necrosis		1 (2%)	1 (2%)	1 (2%)
Transitional epithelium, hyperplasia				1 (2%)
Transitional epithelium, necrosis				1 (2%)
Vomeronasal organ, necrosis		1 (2%)	2 (4%)	3 (6%)
Trachea	(50)	(50)	(50)	(50)
Necrosis				1 (2%)
Special Senses System				
Ear	(1)	(0)	(0)	(0)
External ear, inflammation	1 (100%)			
External ear, necrosis	1 (100%)			
Eye	(50)	(50)	(50)	(50)
Cornea, inflammation	3 (6%)	1 (2%)	4 (8%)	
Lens, cataract			1 (2%)	
Optic nerve, atrophy		1 (2%)		
Harderian gland	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	
Hyperplasia	1 (2%)	4 (8%)	1 (2%)	1 (2%)
Zymbal's gland	(1)	(0)	(0)	(0)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet	1 (2%)			
Infarct	3 (6%)	2 (4%)		2 (4%)
Inflammation	2 (4%)	1 (2%)		
Mineralization	5 (10%)	7 (14%)	9 (18%)	6 (12%)
Nephropathy	39 (78%)	41 (82%)	43 (86%)	37 (74%)
Pigmentation			2 (4%)	
Cortex, cyst	3 (6%)	2 (4%)	1 (2%)	2 (4%)
Papilla, necrosis	1 (2%)	1 (2%)		
Pelvis, dilatation	2 (4%)		1 (2%)	1 (2%)
Renal tubule, dilatation				1 (2%)
Renal tubule, hyperplasia				1 (2%)
Renal tubule, necrosis			2 (4%)	1 (2%)
Ureter	(1)	(0)	(0)	(0)
Inflammation	1 (100%)			
Necrosis	1 (100%)			
Urethra	(0)	(1)	(0)	(0)
Inflammation		1 (100%)		
Necrosis		1 (100%)		
Urinary bladder	(50)	(50)	(50)	(50)
Calculus gross observation				2 (4%)
Inflammation				1 (2%)
Transitional epithelium, hyperplasia				1 (2%)

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR GAVAGE STUDY OF *N*,*N*-DIMETHYL-*p*-TOLUIDINE

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine[a]

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths		1	1	2
Moribund	3	1	7	10
Natural deaths	4	8	3	6
Survivors				
Terminal kill	43	40	39	32
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(50)	(50)	(49)	(49)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Hemangioma	1 (2%)	1 (2%)	1 (2%)	
Hemangiosarcoma		1 (2%)	1 (2%)	
Hepatoblastoma		1 (2%)		4 (8%)
Hepatocellular adenoma	15 (30%)	13 (26%)	8 (16%)	9 (18%)
Hepatocellular adenoma, multiple	2 (4%)	6 (12%)	29 (58%)	35 (70%)
Hepatocellular carcinoma	5 (10%)	10 (20%)	13 (26%)	12 (24%)
Hepatocellular carcinoma, multiple	1 (2%)	3 (6%)	5 (10%)	19 (38%)
Sarcoma, metastatic, skeletal muscle		1 (2%)		
Mesentery	(3)	(8)	(9)	(6)
Sarcoma		1 (13%)		
Sarcoma, metastatic, skeletal muscle		1 (13%)		
Pancreas	(50)	(50)	(50)	(50)
Sarcoma, metastatic, skeletal muscle		1 (2%)		
Salivary glands	(50)	(50)	(50)	(48)
Adenoma	1 (2%)			
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell carcinoma		1 (2%)		
Squamous cell papilloma	1 (2%)	4 (8%)	5 (10%)	7 (14%)
Squamous cell papilloma, multiple		1 (2%)	1 (2%)	
Stomach, glandular	(50)	(50)	(50)	(50)
Adenoma		1 (2%)		
Tongue	(0)	(0)	(0)	(1)
Tooth	(13)	(10)	(7)	(4)
Cardiovascular System				
Blood vessel	(50)	(49)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Sarcoma, metastatic, skeletal muscle		1 (2%)		

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	2 (4%)			2 (4%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Parathyroid gland	(48)	(38)	(38)	(34)
Pituitary gland	(48)	(50)	(50)	(49)
Pars distalis, adenoma		2 (4%)	1 (2%)	
Pars intermedia, adenoma		3 (6%)		
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, adenoma				1 (2%)
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(50)	(49)
Ovary	(50)	(49)	(50)	(50)
Cystadenoma	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Luteoma		1 (2%)		
Thecoma benign		1 (2%)		
Tubulostromal adenoma	1 (2%)			
Oviduct	(0)	(1)	(0)	(0)
Sarcoma, metastatic, skeletal muscle		1 (100%)		
Uterus	(50)	(50)	(50)	(50)
Carcinoma			1 (2%)	
Fibrous histiocytoma			1 (2%)	
Polyp stromal		2 (4%)	2 (4%)	
Sarcoma stromal			1 (2%)	
Squamous cell carcinoma			1 (2%)	
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(49)
Lymph node	(7)	(3)	(5)	(4)
Mediastinal, osteosarcoma, metastatic, bone				1 (25%)
Lymph node, mandibular	(50)	(50)	(50)	(48)
Hemangioma				1 (2%)
Lymph node, mesenteric	(49)	(49)	(49)	(50)
Sarcoma, metastatic, skeletal muscle		1 (2%)		
Spleen	(49)	(49)	(49)	(50)
Hemangiosarcoma		1 (2%)		
Sarcoma, metastatic, skeletal muscle		1 (2%)		
Thymus	(50)	(50)	(48)	(48)
Sarcoma, metastatic, skeletal muscle		1 (2%)		

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Carcinoma	1 (2%)		1 (2%)	
Skin	(50)	(50)	(50)	(50)
Keratoacanthoma	1 (2%)			
Dermis, fibrous histiocytoma		1 (2%)		
Subcutaneous tissue, fibrosarcoma	1 (2%)		1 (2%)	
Subcutaneous tissue, fibrosarcoma, multiple	1 (2%)			
Subcutaneous tissue, liposarcoma		1 (2%)		
Subcutaneous tissue, melanoma malignant			1 (2%)	
Subcutaneous tissue, neurofibrosarcoma			1 (2%)	
Subcutaneous tissue, sarcoma			1 (2%)	
Subcutaneous tissue, schwannoma malignant			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma				1 (2%)
Skeletal muscle	(0)	(2)	(1)	(2)
Rhabdomyosarcoma				2 (100%)
Rhabdomyosarcoma, multiple			1 (100%)	
Sarcoma		1 (50%)		
Nervous System				
Brain	(50)	(50)	(50)	(49)
Meningioma benign				1 (2%)
Peripheral nerve	(0)	(0)	(1)	(0)
Spinal cord	(0)	(0)	(1)	(0)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma		3 (6%)	7 (14%)	12 (24%)
Alveolar/bronchiolar adenoma, multiple	2 (4%)	1 (2%)	1 (2%)	
Alveolar/bronchiolar carcinoma		1 (2%)	2 (4%)	1 (2%)
Carcinoma, metastatic, harderian gland		1 (2%)	2 (4%)	
Fibrous histiocytoma			1 (2%)	
Hepatoblastoma, metastatic, liver				1 (2%)
Hepatocellular carcinoma, metastatic, liver	2 (4%)	3 (6%)		2 (4%)
Osteosarcoma, metastatic, bone				1 (2%)
Sarcoma, metastatic, skeletal muscle		1 (2%)		
Nose	(50)	(49)	(50)	(50)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(49)
Harderian gland	(50)	(50)	(50)	(49)
Adenoma	1 (2%)	6 (12%)	1 (2%)	3 (6%)
Carcinoma		1 (2%)	2 (4%)	

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Sarcoma		1 (2%)		
Urinary bladder	(50)	(50)	(50)	(50)
Fibrous histiocytoma			1 (2%)	
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		2 (4%)	3 (6%)	1 (2%)
Lymphoma malignant	15 (30%)	9 (18%)	11 (22%)	8 (16%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	35	41	47	46
Total primary neoplasms	52	82	107	120
Total animals with benign neoplasms	24	31	40	44
Total benign neoplasms	28	47	57	72
Total animals with malignant neoplasms	21	25	34	36
Total malignant neoplasms	24	35	50	48
Total animals with metastatic neoplasms	2	4	2	4
Total metastatic neoplasms	2	13	2	5

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Harderian Gland: Adenoma				
Overall rate[a]	1/50 (2%)	6/50 (12%)	1/50 (2%)	3/50 (6%)
Adjusted rate[b]	2.1%	13.0%	2.2%	7.2%
Terminal rate[c]	1/43 (2%)	6/40 (15%)	1/39 (3%)	1/32 (3%)
First incidence (days)	729 (T)	729 (T)	729 (T)	578
Poly-3 test[d]	P=0.539	P=0.051	P=0.750	P=0.259
Harderian Gland: Adenoma or Carcinoma				
Overall rate	1/50 (2%)	6/50 (12%)	3/50 (6%)	3/50 (6%)
Adjusted rate	2.1%	13.0%	6.6%	7.2%
Terminal rate	1/43 (2%)	6/40 (15%)	1/39 (3%)	1/32 (3%)
First incidence (days)	729 (T)	729 (T)	669	578
Poly-3 test	P=0.529	P=0.051	P=0.290	P=0.259
Liver: Hepatocellular Adenoma				
Overall rate	17/50 (34%)	19/50 (38%)	37/50 (74%)	44/50 (88%)
Adjusted rate	35.5%	41.1%	80.0%	96.1%
Terminal rate	16/43 (37%)	17/40 (43%)	34/39 (87%)	31/32 (97%)
First incidence (days)	698	720	649	481
Poly-3 test	P<0.001	P=0.364	P<0.001	P<0.001
Liver: Hepatocellular Carcinoma				
Overall rate	6/50 (12%)	13/50 (26%)	18/50 (36%)	31/50 (62%)
Adjusted rate	12.5%	28.2%	39.3%	71.9%
Terminal rate	4/43 (9%)	12/40 (30%)	16/39 (41%)	24/32 (75%)
First incidence (days)	666	720	669	512
Poly-3 test	P<0.001	P=0.049	P=0.002	P<0.001
Liver: Hepatocellular Adenoma or Hepatocellular Carcinoma				
Overall rate	20/50 (40%)	25/50 (50%)	42/50 (84%)	45/50 (90%)
Adjusted rate	41.6%	54.1%	90.6%	98.3%
Terminal rate	18/43 (42%)	23/40 (58%)	38/39 (97%)	32/32 (100%)
First incidence (days)	666	720	649	481
Poly-3 test	P<0.001	P=0.154	P<0.001	P<0.001
Liver: Hepatoblastoma				
Overall rate	0/50 (0%)	1/50 (2%)	0/50 (0%)	4/50 (8%)
Adjusted rate	0.0%	2.2%	0.0%	9.7%
Terminal rate	0/43 (0%)	1/40 (3%)	0/39 (0%)	3/32 (9%)
First incidence (days)	—[e]	729 (T)	—	699
Poly-3 test	P=0.007	P=0.493	—[f]	P=0.044
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	6/50 (12%)	14/50 (28%)	18/50 (36%)	32/50 (64%)
Adjusted rate	12.5%	30.3%	39.3%	74.2%
Terminal rate	4/43 (9%)	13/40 (33%)	16/39 (41%)	25/32 (78%)
First incidence (days)	666	720	669	512
Poly-3 test	P<0.001	P=0.029	P=0.002	P<0.001
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	20/50 (40%)	26/50 (52%)	42/50 (84%)	45/50 (90%)
Adjusted rate	41.6%	56.3%	90.6%	98.3%
Terminal rate	18/43 (42%)	24/40 (60%)	38/39 (97%)	32/32 (100%)
First incidence (days)	666	720	649	481
Poly-3 test	P<0.001	P=0.108	P<0.001	P<0.001

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	2/50 (4%)	4/50 (8%)	8/50 (16%)	12/50 (24%)
Adjusted rate	4.2%	8.7%	17.5%	28.2%
Terminal rate	2/43 (5%)	4/40 (10%)	7/39 (18%)	8/32 (25%)
First incidence (days)	729 (T)	729 (T)	649	570
Poly-3 test	P<0.001	P=0.322	P=0.039	P<0.001
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	2/50 (4%)	5/50 (10%)	9/50 (18%)	13/50 (26%)
Adjusted rate	4.2%	10.8%	19.6%	30.3%
Terminal rate	2/43 (5%)	5/40 (13%)	7/39 (18%)	8/32 (25%)
First incidence (days)	729 (T)	729 (T)	649	570
Poly-3 test	P<0.001	P=0.203	P=0.021	P<0.001
Pituitary Gland (Pars Intermedia): Adenoma				
Overall rate	0/48 (0%)	3/50 (6%)	0/50 (0%)	0/49 (0%)
Adjusted rate	0.0%	6.5%	0.0%	0.0%
Terminal rate	0/41 (0%)	3/40 (8%)	0/39 (0%)	0/32 (0%)
First incidence (days)	—	729 (T)	—	—
Poly-3 test	P=0.279N	P=0.120	—	—
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	1/50 (2%)	5/50 (10%)	6/50 (12%)	7/50 (14%)
Adjusted rate	2.1%	10.8%	13.2%	17.1%
Terminal rate	1/43 (2%)	5/40 (13%)	5/39 (13%)	6/32 (19%)
First incidence (days)	729 (T)	729 (T)	703	708
Poly-3 test	P=0.037	P=0.094	P=0.049	P=0.017
Stomach (Forestomach): Squamous Cell Papilloma or Squamous Cell Carcinoma				
Overall rate	1/50 (2%)	6/50 (12%)	6/50 (12%)	7/50 (14%)
Adjusted rate	2.1%	13.0%	13.2%	17.1%
Terminal rate	1/43 (2%)	6/40 (15%)	5/39 (13%)	6/32 (19%)
First incidence (days)	729 (T)	729 (T)	703	708
Poly-3 test	P=0.055	P=0.051	P=0.049	P=0.017
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	0/50 (0%)	2/50 (4%)	3/50 (6%)	0/50 (0%)
Adjusted rate	0.0%	4.3%	6.6%	0.0%
Terminal rate	0/43 (0%)	2/40 (5%)	3/39 (8%)	0/32 (0%)
First incidence (days)	—	729 (T)	729 (T)	—
Poly-3 test	P=0.456N	P=0.230	P=0.110	—
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	1/50 (2%)	3/50 (6%)	2/50 (4%)	1/50 (2%)
Adjusted rate	2.1%	6.5%	4.4%	2.4%
Terminal rate	0/43 (0%)	3/40 (8%)	2/39 (5%)	1/32 (3%)
First incidence (days)	698	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.483N	P=0.292	P=0.482	P=0.725
All Organs: Histiocytic Sarcoma				
Overall rate	0/50 (0%)	2/50 (4%)	3/50 (6%)	1/50 (2%)
Adjusted rate	0.0%	4.3%	6.5%	2.4%
Terminal rate	0/43 (0%)	0/40 (0%)	0/39 (0%)	1/32 (3%)
First incidence (days)	—	449	585	729 (T)
Poly-3 test	P=0.530	P=0.234	P=0.114	P=0.470

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
All Organs: Malignant Lymphoma				
Overall rate	15/50 (30%)	9/50 (18%)	11/50 (22%)	8/50 (16%)
Adjusted rate	30.7%	19.4%	23.7%	18.9%
Terminal rate	13/43 (30%)	8/40 (20%)	8/39 (21%)	4/32 (13%)
First incidence (days)	444	671	627	570
Poly-3 test	P=0.216N	P=0.151N	P=0.297N	P=0.147N
All Organs: Benign Neoplasms				
Overall rate	24/50 (48%)	31/50 (62%)	40/50 (80%)	44/50 (88%)
Adjusted rate	50.1%	67.1%	85.4%	96.1%
Terminal rate	23/43 (54%)	29/40 (73%)	35/39 (90%)	31/32 (97%)
First incidence (days)	698	720	585	481
Poly-3 test	P<0.001	P=0.068	P<0.001	P<0.001
All Organs: Malignant Neoplasms				
Overall rate	21/50 (42%)	25/50 (50%)	34/50 (68%)	36/50 (72%)
Adjusted rate	42.4%	51.7%	71.8%	80.9%
Terminal rate	16/43 (37%)	19/40 (48%)	26/39 (67%)	26/32 (81%)
First incidence (days)	444	449	585	386
Poly-3 test	P<0.001	P=0.236	P=0.002	P<0.001
All Organs: Benign or Malignant Neoplasms				
Overall rate	35/50 (70%)	41/50 (82%)	47/50 (94%)	46/50 (92%)
Adjusted rate	70.7%	84.8%	99.2%	98.6%
Terminal rate	30/43 (70%)	34/40 (85%)	39/39 (100%)	32/32 (100%)
First incidence (days)	444	449	585	386
Poly-3 test	P<0.001	P=0.072	P<0.001	P<0.001

(T) Terminal kill

[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, and pituitary gland; for other tissues, denominator is number of animals necropsied.

[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[c] Observed incidence at terminal kill

[d] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in a dose group is indicated by **N**.

[e] Not applicable; no neoplasms in animal group

[f] Value of statistic cannot be computed.

TABLE D3a
Historical Incidence of Liver Neoplasms in Control Female B6C3F1/N Mice[a]

Study (Study Start)	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Hepatocellular Carcinoma
Historical Incidence: Corn Oil Gavage Studies			
N,*N*-Dimethyl-*p*-toluidine (October 2004)	17/50	6/50	20/50
Ginkgo biloba extract (March 2005)	17/50	9/50	20/50
Isoeugenol (May 2002)	11/49	3/49	13/49
Kava kava extract (August 2004)	8/50	3/50	10/50
β-Myrcene (April 2002)	6/50	1/50	7/50
Pulegone (April 2003)	13/49	5/49	17/49
3,3′,4,4′-Tetrachloroazobenzene (February 2003)	3/49	2/49	4/49
Total (%)	75/347 (21.6%)	29/347 (8.4%)	91/347 (26.2%)
Mean ± standard deviation	21.6% ± 10.8%	8.3% ± 5.5%	26.2% ± 12.7%
Range	6%-34%	2%-18%	8%-40%
Overall Historical Incidence: All Routes			
Total (%)	380/1,195 (31.8%)	144/1,195 (12.1%)	444/1,195 (37.2%)
Mean ± standard deviation	31.8% ± 21.4%	12.1% ± 10.8%	37.2% ± 22.9%
Range	2%-78%	0%-46%	6%-82%

	Hepatoblastoma	Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma
Historical Incidence: Corn Oil Gavage Studies		
N,*N*-Dimethyl-*p*-toluidine (October 2004)	0/50	20/50
Ginkgo biloba extract (March 2005)	1/50	20/50
Isoeugenol (May 2002)	0/49	13/49
Kava kava extract (August 2004)	0/50	10/50
β-Myrcene (April 2002)	0/50	7/50
Pulegone (April 2003)	0/49	17/49
3,3′,4,4′-Tetrachloroazobenzene (February 2003)	0/49	4/49
Total (%)	1/347 (0.3%)	91/347 (26.2%)
Mean ± standard deviation	0.3% ± 0.8%	26.2% ± 12.7%
Range	0%-2%	8%-40%
Overall Historical Incidence: All Routes		
Total (%)	4/1,195 (0.3%)	444/1,195 (37.2%)
Mean ± standard deviation	0.3% ± 0.8%	37.2% ± 22.9%
Range	0%-2%	6%-82%

[a] Data as of May 2011

TABLE D3b
Historical Incidence of Alveolar/bronchiolar Neoplasms in Control Female B6C3F1/N Mice[a]

Study (Study Start)	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Corn Oil Gavage Studies			
N,*N*-Dimethyl-*p*-toluidine (October 2004)	2/50	0/50	2/50
Ginkgo biloba extract (March 2005)	0/50	1/50	1/50
Isoeugenol (May 2002)	4/48	0/48	4/48
Kava kava extract (August 2004)	2/50	2/50	4/50
β-Myrcene (April 2002)	4/50	2/50	6/50
Pulegone (April 2003)	1/49	2/49	3/49
3,3′,4,4′-Tetrachloroazobenzene (February 2003)	3/49	0/49	3/49
Total (%)	16/346 (4.6%)	7/346 (2.0%)	23/346 (6.7%)
Mean ± standard deviation	4.6% ± 3.1%	2.0% ± 2.0%	6.7% ± 3.2%
Range	0%-8%	0%-4%	2%-12%
Overall Historical Incidence: All Routes			
Total (%)	60/1,196 (5.0%)	44/1,196 (3.7%)	100/1,196 (8.4%)
Mean ± standard deviation	5.0% ± 3.6%	3.7% ± 3.3%	8.4% ± 4.3%
Range	0%-12%	0%-14%	2%-22%

[a] Data as of May 2011

TABLE D3c
Historical Incidence of Squamous Cell Neoplasms of the Forestomach in Control Female B6C3F1/N Mice[a]

Study (Study Start)	Papilloma	Carcinoma	Papilloma or Carcinoma
Historical Incidence: Corn Oil Gavage Studies			
N,*N*-Dimethyl-*p*-toluidine (October 2004)	1/50	0/50	1/50
Ginkgo biloba extract (March 2005)	2/50	0/50	2/50
Isoeugenol (May 2002)	1/49	0/49	1/49
Kava kava extract (August 2004)	3/50	0/50	3/50
β-Myrcene (April 2002)	1/50	0/50	1/50
Pulegone (April 2003)	2/49	0/49	2/49
3,3′,4,4′-Tetrachloroazobenzene (February 2003)	2/50	0/50	2/50
Total (%)	12/348 (3.5%)	0/348	12/348 (3.5%)
Mean ± standard deviation	3.5% ± 1.5%		3.5% ± 1.5%
Range	2%-6%		2%-6%
Overall Historical Incidence: All Routes			
Total (%)	22/1,198 (1.8%)	1/1,198 (0.1%)	23/1,198 (1.9%)
Mean ± standard deviation	1.8% ± 1.7%	0.1% ± 0.4%	1.9% ± 1.6%
Range	0%-6%	0%-2%	0%-6%

[a] Data as of May 2011

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine[a]

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths		1	1	2
Moribund	3	1	7	10
Natural deaths	4	8	3	6
Survivors				
Terminal kill	43	40	39	32
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Foreign body				1 (2%)
Perforation			1 (2%)	1 (2%)
Epithelium, inflammation				1 (2%)
Muscularis, degeneration			1 (2%)	1 (2%)
Muscularis, inflammation	1 (2%)			
Periesophageal tissue, hemorrhage			1 (2%)	
Gallbladder	(50)	(50)	(49)	(49)
Intestine large, cecum	(50)	(50)	(50)	(50)
Fibrosis		1 (2%)		
Lymphoid tissue, hyperplasia, lymphoid	1 (2%)			
Intestine large, colon	(50)	(50)	(50)	(50)
Fibrosis		1 (2%)		
Intestine large, rectum	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Fibrosis		1 (2%)		
Intestine small, jejunum	(50)	(50)	(50)	(50)
Fibrosis		1 (2%)		
Peyer's patch, hyperplasia, lymphoid		1 (2%)		
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)			
Basophilic focus	7 (14%)	5 (10%)	9 (18%)	11 (22%)
Clear cell focus		2 (4%)	2 (4%)	3 (6%)
Eosinophilic focus	20 (40%)	18 (36%)	45 (90%)	38 (76%)
Fatty change	1 (2%)			8 (16%)
Hematopoietic cell proliferation	2 (4%)	4 (8%)	2 (4%)	3 (6%)
Inflammation, chronic active	39 (78%)	27 (54%)	33 (66%)	35 (70%)
Mineralization				1 (2%)
Mixed cell focus	3 (6%)	9 (18%)	7 (14%)	7 (14%)
Necrosis	1 (2%)	8 (16%)	4 (8%)	10 (20%)
Pigmentation	1 (2%)	1 (2%)	1 (2%)	4 (8%)
Bile duct, cyst	2 (4%)	1 (2%)	1 (2%)	3 (6%)
Hepatocyte, hypertrophy		11 (22%)	10 (20%)	17 (34%)
Kupffer cell, hyperplasia		1 (2%)		1 (2%)
Oval cell, hyperplasia				2 (4%)
Serosa, fibrosis		1 (2%)		
Serosa, inflammation, chronic active		1 (2%)		
Mesentery	(3)	(8)	(9)	(6)
Inflammation, chronic		1 (13%)		
Fat, necrosis	3 (100%)	5 (63%)	9 (100%)	6 (100%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Alimentary System (continued)				
Pancreas	(50)	(50)	(50)	(50)
Atrophy	1 (2%)		2 (4%)	
Acinus, hyperplasia				1 (2%)
Acinus, necrosis				1 (2%)
Duct, cyst			2 (4%)	
Salivary glands	(50)	(50)	(50)	(48)
Atrophy				1 (2%)
Fibrosis				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Erosion		1 (2%)		2 (4%)
Fibrosis		1 (2%)		
Inflammation	3 (6%)	4 (8%)	7 (14%)	16 (32%)
Necrosis	1 (2%)			
Ulcer	2 (4%)	2 (4%)	4 (8%)	7 (14%)
Epithelium, cyst		1 (2%)		1 (2%)
Epithelium, hyperplasia	3 (6%)	5 (10%)	12 (24%)	17 (34%)
Stomach, glandular	(50)	(50)	(50)	(50)
Mineralization	1 (2%)			
Epithelium, necrosis	1 (2%)			
Glands, dysplasia				1 (2%)
Tongue	(0)	(0)	(0)	(1)
Cyst				1 (100%)
Tooth	(13)	(10)	(7)	(4)
Dysplasia	13 (100%)	10 (100%)	4 (57%)	4 (100%)
Peridontal tissue, pulp, inflammation	1 (8%)			
Cardiovascular System				
Blood vessel	(50)	(49)	(50)	(50)
Embolus bacterial		1 (2%)		
Inflammation				3 (6%)
Media, pulmonary artery, hyperplasia		1 (2%)		
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	5 (10%)	2 (4%)	4 (8%)	2 (4%)
Inflammation				1 (2%)
Mineralization	1 (2%)	2 (4%)	1 (2%)	5 (10%)
Necrosis			2 (4%)	
Epicardium, fibrosis				1 (2%)
Valve, thrombosis		1 (2%)	1 (2%)	
Ventricle, thrombosis			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)		
Necrosis			2 (4%)	
Vacuolization cytoplasmic			1 (2%)	
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	3 (6%)			
Necrosis			1 (2%)	
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)		2 (4%)
Parathyroid gland	(48)	(38)	(38)	(34)
Pituitary gland	(48)	(50)	(50)	(49)
Pars distalis, angiectasis				1 (2%)
Pars distalis, hyperplasia	5 (10%)	3 (6%)	3 (6%)	3 (6%)
Pars intermedia, hyperplasia	1 (2%)	1 (2%)	1 (2%)	2 (4%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Endocrine System (continued)				
Thyroid gland	(50)	(50)	(50)	(50)
Atrophy		2 (4%)		
Inflammation		1 (2%)	1 (2%)	
Follicle, degeneration			1 (2%)	
Follicular cell, hyperplasia		1 (2%)		
Follicular cell, hypertrophy			1 (2%)	
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(50)	(49)
Ovary	(50)	(49)	(50)	(50)
Angiectasis	2 (4%)		1 (2%)	1 (2%)
Atrophy	40 (80%)	43 (88%)	40 (80%)	45 (90%)
Cyst	4 (8%)	6 (12%)	4 (8%)	2 (4%)
Hemorrhage	2 (4%)		1 (2%)	
Inflammation		2 (4%)		
Thrombosis	2 (4%)			3 (6%)
Oviduct	(0)	(1)	(0)	(0)
Uterus	(50)	(50)	(50)	(50)
Angiectasis	2 (4%)	2 (4%)	1 (2%)	
Atrophy				1 (2%)
Dilatation	13 (26%)	13 (26%)	6 (12%)	10 (20%)
Inflammation	1 (2%)	3 (6%)	2 (4%)	
Thrombosis		1 (2%)	1 (2%)	
Endometrium, hyperplasia, cystic	25 (50%)	17 (34%)	11 (22%)	9 (18%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(49)
Atrophy	1 (2%)	2 (4%)	2 (4%)	3 (6%)
Hyperplasia	5 (10%)	14 (28%)	15 (30%)	14 (29%)
Lymph node	(7)	(3)	(5)	(4)
Lumbar, hemorrhage				1 (25%)
Mediastinal, hyperplasia, lymphoid			1 (20%)	
Renal, ectasia	2 (29%)			
Renal, hemorrhage		1 (33%)		
Lymph node, mandibular	(50)	(50)	(50)	(48)
Atrophy	1 (2%)	4 (8%)	5 (10%)	5 (10%)
Hyperplasia, lymphoid	3 (6%)	5 (10%)		3 (6%)
Hyperplasia, plasma cell		1 (2%)		
Lymph node, mesenteric	(49)	(49)	(49)	(50)
Angiectasis	1 (2%)			
Atrophy	1 (2%)	5 (10%)	5 (10%)	12 (24%)
Hyperplasia, lymphoid	7 (14%)	3 (6%)	1 (2%)	
Infiltration cellular, plasma cell			1 (2%)	
Inflammation, granulomatous				1 (2%)
Necrosis				1 (2%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Hematopoietic System (continued)				
Spleen	(49)	(49)	(49)	(50)
Atrophy	3 (6%)	8 (16%)	1 (2%)	6 (12%)
Hematopoietic cell proliferation	18 (37%)	23 (47%)	24 (49%)	21 (42%)
Hyperplasia, lymphoid	14 (29%)	15 (31%)	12 (24%)	15 (30%)
Infarct		1 (2%)		
Infiltration cellular, plasma cell	1 (2%)			
Pigmentation	37 (76%)	39 (80%)	33 (67%)	43 (86%)
Capsule, fibrosis		1 (2%)		
Red pulp, atrophy				5 (10%)
Thymus	(50)	(50)	(48)	(48)
Atrophy	46 (92%)	46 (92%)	39 (81%)	43 (90%)
Hyperplasia, histiocytic		1 (2%)		
Hyperplasia, lymphoid	3 (6%)			
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Skin	(50)	(50)	(50)	(50)
Hemorrhage			1 (2%)	
Inflammation	1 (2%)	2 (4%)		1 (2%)
Ulcer		2 (4%)	2 (4%)	1 (2%)
Dermis, fibrosis		1 (2%)	1 (2%)	
Epidermis, hyperplasia	1 (2%)		2 (4%)	
Sebaceous gland, hyperplasia			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibro-osseous lesion	3 (6%)	5 (10%)	6 (12%)	11 (22%)
Fracture				1 (2%)
Osteopetrosis	1 (2%)	1 (2%)		1 (2%)
Skeletal muscle	(0)	(2)	(1)	(2)
Inflammation		1 (50%)		
Nervous System				
Brain	(50)	(50)	(50)	(49)
Necrosis	1 (2%)		1 (2%)	
Olfactory lobe, atrophy				8 (16%)
Peripheral nerve	(0)	(0)	(1)	(0)
Spinal cord	(0)	(0)	(1)	(0)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Foreign body		1 (2%)		2 (4%)
Hemorrhage			1 (2%)	
Inflammation		2 (4%)		2 (4%)
Alveolar epithelium, hyperplasia	2 (4%)	3 (6%)	8 (16%)	2 (4%)
Alveolus, infiltration cellular, histiocyte	1 (2%)			7 (14%)
Bronchiole, epithelium, necrosis				1 (2%)
Bronchiole, epithelium, regeneration				5 (10%)
Bronchus, necrosis				5 (10%)
Bronchus, epithelium, regeneration				5 (10%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Respiratory System (continued)				
Nose	(50)	(49)	(50)	(50)
Inflammation	3 (6%)	7 (14%)	3 (6%)	32 (64%)
Glands, lateral wall, dilatation				2 (4%)
Glands, olfactory epithelium, dilatation	13 (26%)	14 (29%)	20 (40%)	46 (92%)
Glands, olfactory epithelium, hyperplasia	2 (4%)	14 (29%)	14 (28%)	50 (100%)
Glands, olfactory epithelium, metaplasia, respiratory	2 (4%)	5 (10%)	7 (14%)	44 (88%)
Glands, respiratory epithelium, dilatation	10 (20%)	17 (35%)	15 (30%)	33 (66%)
Glands, respiratory epithelium, hyperplasia		2 (4%)	12 (24%)	13 (26%)
Glands, respiratory epithelium, metaplasia, respiratory			10 (20%)	10 (20%)
Nasolacrimal duct, hyperplasia, regenerative				4 (8%)
Nerve, atrophy				41 (82%)
Olfactory epithelium, accumulation, hyaline droplet	2 (4%)	5 (10%)	8 (16%)	15 (30%)
Olfactory epithelium, degeneration				1 (2%)
Olfactory epithelium, metaplasia, respiratory	1 (2%)	6 (12%)	14 (28%)	46 (92%)
Olfactory epithelium, necrosis			3 (6%)	6 (12%)
Respiratory epithelium, accumulation, hyaline droplet	33 (66%)	34 (69%)	39 (78%)	36 (72%)
Respiratory epithelium, hyperplasia	11 (22%)	15 (31%)	11 (22%)	30 (60%)
Respiratory epithelium, hyperplasia, regenerative				3 (6%)
Respiratory epithelium, necrosis				5 (10%)
Transitional epithelium, hyperplasia, regenerative				1 (2%)
Transitional epithelium, necrosis				2 (4%)
Vomeronasal organ, necrosis				4 (8%)
Trachea	(50)	(50)	(50)	(50)
Inflammation				1 (2%)
Glands, hyperplasia				1 (2%)
Special Senses System				
Eye	(50)	(50)	(50)	(49)
Fibrosis			1 (2%)	
Cornea, inflammation		1 (2%)	1 (2%)	3 (6%)
Lens, cataract			1 (2%)	
Optic nerve, atrophy			1 (2%)	
Harderian gland	(50)	(50)	(50)	(49)
Fibrosis		1 (2%)		
Hyperplasia	4 (8%)	2 (4%)		2 (4%)
Inflammation	1 (2%)			

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet		2 (4%)	1 (2%)	
Infarct	4 (8%)	3 (6%)	4 (8%)	
Inflammation			1 (2%)	2 (4%)
Mineralization		1 (2%)		3 (6%)
Nephropathy	13 (26%)	15 (30%)	15 (30%)	17 (34%)
Cortex, cyst				1 (2%)
Papilla, necrosis				1 (2%)
Renal tubule, necrosis	1 (2%)		2 (4%)	2 (4%)
Urinary bladder	(50)	(50)	(50)	(50)

APPENDIX E
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

BACTERIAL MUTAGENICITY TEST PROTOCOL

Testing procedures used at BioReliance Corporation followed protocols reported by Zeiger *et al.* (1992); in the tests conducted at SITEK Research Laboratories, using the same chemical lot that was tested in the 2-year studies (050404), a slightly modified procedure was used, and that is described in more detail below. *N,N*-Dimethyl-*p*-toluidine was tested as a coded sample. In the tests conducted at BioReliance Corporation, it was incubated with the *Salmonella typhimurium* tester strains TA97, TA98, TA100, and TA1535 either in buffer or 10% or 30% S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

The protocol used at SITEK Research Laboratories used only 10% rat liver S9 for exogenous metabolic activation, and employed *Escherichia coli* strain WP2 *uvrA*/pKM101 as a bacterial tester strain in addition to *S. typhimurium* strains TA98 and TA100. Incubation of bacterial strains with *N,N*-dimethyl-*p*-toluidine and subsequent plating were carried out as described above.

In both studies, each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of *N,N*-dimethyl-*p*-toluidine. The high dose was limited by toxicity. All trials were repeated using the same or higher S9 concentration.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that is not dose related, is not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment. There is no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive, although positive calls are typically reserved for increases in mutant colonies that are at least twofold over background.

MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOLS

Slide-Based Assay

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 3-month gavage study (lot H3124A), peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were shipped to the genetic toxicity testing laboratory where they were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs, mature erythrocytes) per animal. In addition, the percentage of polychromatic erythrocytes (PCEs, reticulocytes) among a population of 1,000 erythrocytes in the peripheral blood was scored for each dose group as a measure of bone marrow toxicity.

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among NCEs was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the control group. In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the slide-based micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dosed group is less than or equal to 0.025 divided by the number of dosed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Results of the 3-month studies were accepted without repeat tests, because additional test data could not be obtained. Ultimately, the final call is

determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

Flow Cytometric Assay

The treatment protocol and blood sample preparation procedures for this assay have been described in detail by Recio *et al*. (2010). Male mice were administered 30, 60, or 75 mg *N*,*N*-dimethyl-*p*-toluidine (lot 050404)/kg body weight in corn oil by gavage once daily for 4 days. The highest dose was based on the toxicity information obtained in the 3-month mouse study. Ethyl methanesulfonate in 0.9% saline was used as the positive control. Four hours after the fourth dose, peripheral blood samples were collected and processed for flow cytometric evaluation of micronucleated erythrocytes as described by Witt *et al*. (2008). For each sample, 20,000 immature $CD71^+$ reticulocytes were analyzed to determine the frequency of micronucleated reticulocytes. More than 10^6 mature erythrocytes were enumerated during the reticulocyte analysis, and the percentage of reticulocytes among total erythrocytes was calculated as a measure of bone marrow toxicity.

Because measurements of micronucleus frequency using flow cytometry are obtained from a large number of cells, it is reasonable to assume that the proportion of micronucleated cells is approximately normally distributed within each sample (Kissling *et al*., 2007). The NTP uses Levene's test at α=0.05 to test for equal variances among the treatment groups. In the case of equal variances, linear regression was used to test for a dose-related trend, and Williams' test (Williams, 1971, 1972) was used to test for pairwise differences between each treatment group and the vehicle control group. In the case of unequal variances, Jonckheere's test (Jonckheere, 1954) was used to test for a linear trend, and pairwise differences with the control group were tested using Dunn's test (Dunn, 1964). To correct for multiple pairwise comparisons, the P value for each comparison was multiplied by the number of comparisons made. Trend tests and pairwise comparisons with the controls were considered statistically significant at $P \leq 0.025$. A one-tailed independent t-test was used to verify a positive response ($P \leq 0.05$) to the control compound, ethyl methanesulfonate.

DNA Damage Test Protocol

The treatment protocol and tissue sample preparation procedures followed in this study have been described in detail (Recio *et al*., 2010). Male mice used in this assay are the same as those used in the flow cytometric micronucleus assay. In addition, male Sprague-Dawley rats were administered 60 mg/kg *N*,*N*-dimethyl-*p*-toluidine (lot 050404) in a 1% acetone/corn oil vehicle by gavage once daily for 4 days; this dose is the same as the highest dose used in the 2-year study in F344/N rats. Four hours after the fourth dose, blood samples were collected from mice and samples of the left liver lobe were collected from mice and rats for assessment of DNA damage using the comet assay (Tice *et al*., 2000; Ghanayem *et al*., 2005; Burlinson *et al*., 2007). Cell preparations were diluted with phosphate buffered saline, mixed with 0.5% low melting point agarose at 37° C, layered onto slides, and placed in cold lysing solution overnight. After rinsing, slides were treated with cold alkali (200 mM NaOH, 1 mM Na_2EDTA, pH>13) for 20 minutes, then electrophoresed at 4° to 10° C for 20 minutes at 1.0 V/cm, 300 mA. Slides were then neutralized for 5 minutes, incubated for 5 minutes in ice-cold 100% ethanol, and allowed to air dry. Slides were stained with SYBR® Gold and 100 cells were scored per tissue per animal using Comet Assay IV Imaging Software Version 4.11 (Perceptive Instruments, Ltd., Suffolk, UK). For each cell, the extent of DNA migration was characterized using the percent tail DNA endpoint measurement (intensity of all tail pixels divided by the total intensity of all pixels in the comet, expressed as a percentage).

For the study conducted in rats, a one-tailed pairwise comparison using Student's t-test was used to assess significance ($P \leq 0.05$) of the percent tail DNA in *N*,*N*-dimethyl-*p*-toluidine-treated rats compared with the vehicle control group. For the study conducted in mice using multiple dose levels, the Shapiro-Wilk test was first used to assess normality of the vehicle control group. Data that were normally distributed were analyzed using an independent Student's t-test to compare each dose level to the concurrent control and linear regression to determine the presence of a dose response. Normally distributed data were also tested for homogeneity of variances using the F test; for data of unequal variances, the Welch's approximation for unequal variances t-test value was used for determination of a one-tailed significant ($P<0.05$) increase in DNA migration. Data that were not normally distributed were analyzed by the Mann-Whitney test (Mann and Whitney, 1947) comparing each dose level to the vehicle control, followed by the Kendall rank correlation test (Kendall, 1938) to determine the presence of a dose response. Trend tests were considered statistically significant at $P \leq 0.025$ and pairwise comparisons were significant

at P≤0.008 (0.025 divided by the number of dosed groups) to correct for multiple comparisons. A one-tailed independent t-test was used to verify a significant (P≤0.05) induction of DNA damage by the positive control compound, ethyl methanesulfonate.

EVALUATION PROTOCOL

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple aliquots of a chemical were tested in the same assay, and different results were obtained among aliquots and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the *in vitro* assays have another variable that must be considered in arriving at an overall test result. *In vitro* assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The summary table in the Abstract of this Technical Report presents a result that represents a scientific judgement of the overall evidence for activity of the chemical in an assay.

RESULTS

N,N-Dimethyl-*p*-toluidine was tested in two independent bacterial gene mutation studies, and negative results were obtained in both studies (Tables E1 and E2). In the first study (concentration range, 0.33 to 1,000 µg/plate), no increases in the numbers of mutant colonies were seen in *S. typhimurium* strains TA97, TA98, TA100, or TA1535, with or without 10% or 30% S9 derived from induced hamster or rat liver. In the second study, which tested the same chemical lot (050404) that was used in the 2-year studies, negative results were obtained over a concentration range of 50 to 1,500 µg/plate in *S. typhimurium* strains TA98 and TA100 and in *E. coli* WP2 *uvrA*/pKM101, with and without 10% rat liver S9.

In vivo, no significant increases in the frequencies of micronucleated erythrocytes, an indicator of chromosomal damage, were observed in peripheral blood of male or female B6C3F1/N mice treated with 15 to 125 mg/kg per day *N,N*-dimethyl-*p*-toluidine by gavage for 3 months (Table E3). No significant alterations in the percentage of circulating reticulocytes were observed, suggesting that *N,N*-dimethyl-*p*-toluidine did not induce bone marrow toxicity over the dose range tested. Results of a second micronucleus test in male B6C3F1/N mice administered 30 to 75 mg/kg *N,N*-dimethyl-*p*-toluidine (lot 050404) by gavage once daily for 4 days were also negative and again, no significant alterations in the percentage of circulating reticulocytes were observed (Table E4).

Two independent comet assays were conducted with *N,N*-dimethyl-*p*-toluidine to measure induction of DNA damage in liver and blood leukocytes. In the first study, conducted in male B6C3F1/N mice, *N,N*-dimethyl-*p*-toluidine administered by gavage over a range of 30 to 75 mg/kg once daily for 4 days did not produce an increase in DNA migration in liver cells or blood leukocytes (Table E5). In the second study, conducted in male Sprague-Dawley rats, *N,N*-dimethyl-*p*-toluidine administered by gavage at a single dose of 60 mg/kg per day for 4 days was associated with a small but statistically significant increase in percent tail DNA in liver cells compared with the vehicle control group (Table E6).

TABLE E1
Mutagenicity of *N,N*-Dimethyl-*p*-toluidine in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% hamster S9	With 30% hamster S9	With 10% rat S9	With 30% rat S9
TA100							
	0	169 ± 7	198 ± 13	180 ± 6	179 ± 10	178 ± 12	183 ± 4
	10	184 ± 12	204 ± 4	176 ± 3	166 ± 21	180 ± 3	136 ± 7
	33	182 ± 14	198 ± 4	188 ± 2	170 ± 13	173 ± 10	170 ± 11
	100	163 ± 16	200 ± 8	187 ± 10	165 ± 18	195 ± 10	177 ± 2
	333	92 ± 10[b]	166 ± 7	154 ± 5	75 ± 3[b]	166 ± 5	79 ± 11[b]
	500	67 ± 9[b]			6 ± 1[b]		52 ± 6[b]
	1,000		Toxic	Toxic		2 ± 2[b]	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[c]		669 ± 22	665 ± 14	2,198 ± 23	736 ± 22	855 ± 85	735 ± 46

		Without S9	Without S9	Without S9
TA97				
	0	114 ± 9	137 ± 12	146 ± 11
	0.33		161 ± 2	
	1		147 ± 7	
	3.3		153 ± 5	
	10	140 ± 8	134 ± 8	143 ± 9
	33	123 ± 12	148 ± 7	146 ± 11
	100	130 ± 9		146 ± 8
	333	110 ± 13		114 ± 14[b]
	500	57 ± 20[b]		
	1,000			Toxic
Trial summary		Equivocal	Equivocal	Negative
Positive control		517 ± 92	699 ± 7	500 ± 54

		With 10% hamster S9	With 30% hamster S9	With 30% hamster S9	With 10% rat S9	With 30% rat S9	With 30% rat S9
TA97 (continued)							
	0	171 ± 9	158 ± 2	146 ± 5	171 ± 8	181 ± 16	219 ± 6
	10	170 ± 11	140 ± 17		163 ± 6	186 ± 15	
	33	157 ± 3	162 ± 3	188 ± 17	163 ± 5	168 ± 9	223 ± 12
	100	149 ± 8	143 ± 13	178 ± 7	157 ± 4	194 ± 15	201 ± 7
	333	146 ± 18[b]	183 ± 8	158 ± 2	133 ± 5[b]	193 ± 3	194 ± 24
	500		188 ± 6	150 ± 2[b]		199 ± 5	164 ± 4
	1,000	Toxic		0 ± 0[b]	23 ± 17[b]		2 ± 0[b]
Trial summary		Negative	Equivocal	Equivocal	Negative	Negative	Negative
Positive control		1,131 ± 7	1,048 ± 77	1,553 ± 54	2,072 ± 50	669 ± 12	664 ± 19

TABLE E1
Mutagenicity of *N,N*-Dimethyl-*p*-toluidine in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% hamster S9	With 30% hamster S9	With 10% rat S9	With 30% rat S9
TA98							
	0	17 ± 1	13 ± 1	24 ± 3	20 ± 3	16 ± 2	23 ± 1
	10	14 ± 3	15 ± 3	22 ± 4	19 ± 3	22 ± 1	22 ± 0
	33	15 ± 2	15 ± 1	27 ± 3	23 ± 2	15 ± 2	23 ± 3
	100	16 ± 2	13 ± 2	25 ± 5	16 ± 1	22 ± 2	24 ± 5
	333	8 ± 0[b]	13 ± 1[b]	13 ± 3[b]	6 ± 1[b]	10 ± 3[b]	11 ± 1[b]
	500	8 ± 1[b]			5 ± 2[b]		8 ± 1[b]
	1,000		Toxic	0 ± 0[b]		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		168 ± 25	167 ± 16	925 ± 23	1,038 ± 42	688 ± 42	251 ± 34

		Without S9	Without S9	With 10% hamster S9	With 30% hamster S9	With 30% hamster S9
TA1535						
	0	22 ± 3	20 ± 1	16 ± 2	18 ± 6	14 ± 1
	10	23 ± 1	17 ± 4	15 ± 2	16 ± 2	
	33	18 ± 2	18 ± 2	13 ± 1	16 ± 1	16 ± 3
	100	20 ± 4	19 ± 1	16 ± 1	16 ± 3	18 ± 2[d]
	333	20 ± 2	13 ± 1[b]	9 ± 1[b]	18 ± 2	17 ± 3
	500	5 ± 1[b]			14 ± 2	11 ± 3
	1,000		Toxic	Toxic		0 ± 0[b]
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		273 ± 8	267 ± 40	192 ± 14	241 ± 16	295 ± 12

		With 10% rat S9	With 30% rat S9	With 30% rat S9
TA1535 (continued)				
	0	15 ± 2	14 ± 2	16 ± 2
	10	12 ± 1	15 ± 2	
	33	12 ± 1	15 ± 2	18 ± 3
	100	13 ± 2	18 ± 2	17 ± 5
	333	12 ± 2[b]	12 ± 2	14 ± 4
	500		15 ± 2	14 ± 1
	1,000	Toxic		1 ± 1[b]
Trial summary		Negative	Negative	Negative
Positive control		339 ± 55	109 ± 17	135 ± 11

[a] Study was performed at BioReliance Corporation. Data are presented as revertants/plate (mean ± standard error) from three plates. The detailed protocol is presented by Zeiger *et al*. (1992). 0 µg/plate was the solvent control.
[b] Slight toxicity
[c] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.
[d] Contamination

TABLE E2
Mutagenicity of *N,N*-Dimethyl-*p*-toluidine in Bacterial Tester Strains[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% rat S9	With 10% rat S9
TA100					
	0	111 ± 7	87 ± 5	83 ± 5	74 ± 3
	50	105 ± 10	87 ± 6		
	100	110 ± 3	83 ± 4	80 ± 2	71 ± 6
	250	104 ± 2	74 ± 9		
	500	61 ± 4	66 ± 8	78 ± 6	68 ± 3
	750	0 ± 0	2 ± 1	46 ± 4	65 ± 1
	1,000			42 ± 4	39 ± 6
	1,500			0 ± 0	1 ± 1
Trial summary		Negative	Negative	Negative	Negative
Positive control[b]		611 ± 11	464 ± 12	925 ± 40	905 ± 19
TA98					
	0	25 ± 1	21 ± 2	34 ± 3	27 ± 2
	50	24 ± 2	23 ± 4		
	100	24 ± 4	19 ± 3	30 ± 1	25 ± 2
	250	24 ± 1	19 ± 2		
	500	31 ± 0	17 ± 1	41 ± 1	27 ± 3
	750	0 ± 0	0 ± 0	28 ± 3	27 ± 4
	1,000			23 ± 4	26 ± 1
	1,500			2 ± 0	5 ± 1
Trial summary		Negative	Negative	Negative	Negative
Positive control		651 ± 31	410 ± 5	1,025 ± 74	757 ± 40
***Escherichia coli* WP2 *uvrA*/pKM101**					
	0	154 ± 6	178 ± 5	208 ± 6	217 ± 5
	50	159 ± 7	222 ± 16		
	100	158 ± 14	237 ± 14	199 ± 10	210 ± 7
	250	122 ± 3	189 ± 5		
	500	103 ± 6	155 ± 4	189 ± 12	193 ± 9
	750	0 ± 0	0 ± 0	187 ± 11	172 ± 8
	1,000			155 ± 8	148 ± 12
	1,500			124 ± 8	118 ± 4
Trial summary		Negative	Negative	Negative	Negative
Positive control		1,406 ± 45	1,912 ± 11	926 ± 40	1,160 ± 33

[a] Study was performed at SITEK Research Laboratories using lot 050404 (same lot used in the 2-year studies). Data are presented as revertants/plate (mean ± standard error) from three plates. 0 µg/plate was the solvent control.

[b] The positive controls in the absence of metabolic activation were sodium azide (TA100), 4-nitro-*o*-phenylenediamine (TA98), and methyl methanesulfonate (*E. coli*). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE E3
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of *N,N*-Dimethyl-*p*-toluidine by Gavage for 3 Months[a]

	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs[b] (%)
Male					
Corn oil[d]	0	5	2.00 ± 0.32		3.34 ± 0.24
N,N-Dimethyl-*p*-toluidine	15	5	2.10 ± 0.29	0.4379	2.62 ± 0.05
	30	5	2.40 ± 0.19	0.2730	3.20 ± 0.25
	60	5	2.80 ± 0.90	0.1238	4.16 ± 0.29
	125	5	3.00 ± 0.52	0.0784	3.94 ± 0.11
			P=0.050[e]		
Female					
Corn oil	0	5	1.50 ± 0.16		4.24 ± 0.36
N,N-Dimethyl-*p*-toluidine	15	5	1.90 ± 0.40	0.2462	3.32 ± 0.29
	30	5	1.70 ± 0.12	0.3617	3.24 ± 0.45
	60	5	1.30 ± 0.41	0.6474	3.58 ± 0.25
	125	5	2.10 ± 0.40	0.1584	5.36 ± 0.60
			P=0.238		

[a] Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor *et al.* (1990). NCE=normochromatic erythrocyte; PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison with the vehicle control group; dosed group values are significant at $P \leq 0.006$
[d] Vehicle control
[e] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test; significant at $P \leq 0.025$

TABLE E4
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Male Mice Following Administration of *N*,*N*-Dimethyl-*p*-toluidine by Gavage for 4 Days[a]

	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated PCEs/ 1,000 PCEs[b]	P Value[c]	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	PCEs[b] (%)	P Value[c]
Corn oil[d]	0	5	2.59 ± 0.20		1.46 ± 0.02		1.270 ± 0.11	
N,*N*-Dimethyl-*p*-toluidine	30	5	2.57 ± 0.19	0.5114	1.49 ± 0.03	0.3095	1.201 ± 0.07	0.748
	60	5	2.66 ± 0.22	0.5200	1.47 ± 0.02	0.3706	1.140 ± 0.15	0.465
	75	5	2.78 ± 0.54	0.4341	1.54 ± 0.04	0.0588	1.103 ± 0.12	0.430
			P=0.327[e]		P=0.089		P=0.243	
Ethyl methanesulfonate[f]	150	5	12.18 ± 0.34	0.0000	1.69 ± 0.04	0.0004	0.942 ± 0.04	0.015

[a] Study was performed at ILS, Inc. The detailed protocol is presented by Witt *et al*. (2008). NCE=normochromatic erythrocyte; PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison with the vehicle control group; values are significant at P≤0.025 by Williams' test
[d] Vehicle control
[e] Significance tested by a linear regression trend test; significant at P≤0.025
[f] Positive control; pairwise comparison with the vehicle control group; values are significant at P≤0.05 by a one-tailed independent t-test

TABLE E5
DNA Damage in the Blood and Liver of Male B6C3F1/N Mice Following Administration of *N,N*-Dimethyl-*p*-Toluidine by Gavage for 4 Days[a]

	Dose (mg/kg)	Number of Animals	Percent Tail DNA[b]	P Value[c]
Blood				
Corn oil[d]	0	5	2.0 ± 0.24	
N,N-Dimethyl-*p*-toluidine	30	5	1.9 ± 0.23	0.549
	60	5	1.5 ± 0.14	0.922
	75	5	2.2 ± 0.30	0.308
			P=0.943[e]	
Ethyl methanesulfonate[f]	150	5	20.7 ± 1.10	<0.001
Liver				
Corn oil	0	5	5.3 ± 0.59	
N,N-Dimethyl-*p*-toluidine	30	5	5.7 ± 1.70	0.411
	60	5	6.5 ± 0.42	0.067
	75	5	6.3 ± 0.81	0.178
			P=0.364	
Ethyl methanesulfonate	150	5	19.2 ± 1.00	<0.001

[a] Study was performed at ILS, Inc. The detailed protocol is presented by Recio *et al.* (2010).
[b] Mean ± standard error
[c] Pairwise comparison with the vehicle control group; dosed group values are significant at P≤0.008 by Student's t-test; positive control values are significant at P≤0.05 by a one-tailed independent t-test.
[d] Vehicle control
[e] Significance of percent tail DNA tested by a linear regression trend test; significant at P≤0.025
[f] Positive control

TABLE E6
DNA Damage in the Liver of Male Sprague-Dawley Rats Following Administration of *N,N*-Dimethyl-*p*-Toluidine by Gavage for 4 Days[a]

	Dose (mg/kg)	Number of Animals	Percent Tail DNA[b]	P Value[c]
1% Acetone/corn oil[d]	0	6	10.5 ± 1.23	
N,N-Dimethyl-*p*-toluidine	60	6	14.6 ± 1.35	0.024
Ethyl methanesulfonate[e]	200	6	35.0 ± 1.05	<0.001

[a] Study was performed at ILS, Inc. The detailed protocol is presented by Recio *et al.* (2010).
[b] Mean ± standard error
[c] Pairwise comparison with the vehicle control group; values are significant at PÆ0.05 by Student's t-test
[d] Vehicle control
[e] Positive control

APPENDIX F
CLINICAL PATHOLOGY RESULTS

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of *N,N*-Dimethyl-*p*-toluidine[a]

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Male						
Hematology						
n						
Day 25	10	10	9	10	8	0[b]
Day 88	10	10	10	10	9	0
Week 14	10	10	10	10	9	0
Hematocrit (%)						
Day 25	49.7 ± 0.3	45.0 ± 0.5**	42.8 ± 0.4**	40.2 ± 0.5**	39.2 ± 0.5**	
Week 14	46.1 ± 0.4	42.1 ± 0.5**	42.3 ± 0.4**	42.1 ± 0.4**	42.4 ± 0.7**	
Hemoglobin (g/dL)						
Day 25	15.3 ± 0.1	13.3 ± 0.1**	12.5 ± 0.1**	11.8 ± 0.1**	11.0 ± 0.1**	
Week 14	14.8 ± 0.1	13.0 ± 0.2**	13.0 ± 0.1**	12.9 ± 0.1**	12.7 ± 0.2**	
Erythrocytes (10^6/μL)						
Day 25	8.26 ± 0.05	7.44 ± 0.07**	6.79 ± 0.07**	5.97 ± 0.09**	5.06 ± 0.05**	
Week 14	8.62 ± 0.07	7.43 ± 0.08**	6.94 ± 0.05**	6.40 ± 0.07**	6.19 ± 0.07**	
Reticulocytes (10^6/μL)						
Day 25	0.26 ± 0.01	0.50 ± 0.01**	0.64 ± 0.01**	0.94 ± 0.03**	1.08 ± 0.03**	
Week 14	0.25 ± 0.01	0.50 ± 0.01**	0.60 ± 0.02**	0.76 ± 0.01**	0.89 ± 0.04**	
Nucleated erythrocytes/100 leukocytes						
Day 25	0.2 ± 0.1	1.3 ± 0.4*	1.3 ± 0.5*	4.7 ± 0.7**	21.6 ± 2.1**	
Week 14	0.2 ± 0.1	0.9 ± 0.2*	2.0 ± 0.4**	1.7 ± 0.3**	3.6 ± 0.6**	
Mean cell volume (fL)						
Day 25	60.2 ± 0.2	60.5 ± 0.2	63.1 ± 0.2**	67.5 ± 0.6**	77.5 ± 0.5**	
Week 14	53.5 ± 0.3	56.6 ± 0.3**	61.1 ± 0.3**	65.8 ± 0.3**	68.5 ± 0.6**	
Mean cell hemoglobin (pg)						
Day 25	18.5 ± 0.1	17.9 ± 0.1	18.4 ± 0.1	19.7 ± 0.1**	21.8 ± 0.1**	
Week 14	17.2 ± 0.1	17.5 ± 0.1*	18.7 ± 0.1**	20.1 ± 0.1**	20.6 ± 0.2**	
Mean cell hemoglobin concentration (g/dL)						
Day 25	30.8 ± 0.1	29.7 ± 0.1**	29.2 ± 0.2**	29.2 ± 0.1**	28.2 ± 0.1**	
Week 14	32.1 ± 0.1	31.0 ± 0.2**	30.7 ± 0.1**	30.5 ± 0.1**	30.0 ± 0.1**	
Platelets (10^3/μL)						
Day 25	930.9 ± 35.0	1,067.8 ± 37.9*	1,077.6 ± 28.2*	1,051.3 ± 21.8	1,097.9 ± 50.0*	
Week 14	666.5 ± 20.3	738.3 ± 28.4	848.6 ± 25.7**	694.9 ± 23.0	735.8 ± 20.0	
Leukocytes (10^3/μL)						
Day 25	12.41 ± 0.37	11.16 ± 0.35	12.09 ± 0.51	10.53 ± 0.41**	8.01 ± 0.18**	
Week 14	8.11 ± 0.37	8.77 ± 0.41	8.53 ± 0.78	7.30 ± 0.37	6.28 ± 0.20**	
Segmented neutrophils (10^3/μL)						
Day 25	1.12 ± 0.08	1.05 ± 0.03	1.67 ± 0.24	1.10 ± 0.05	1.31 ± 0.11	
Week 14	1.28 ± 0.11	1.06 ± 0.09	1.10 ± 0.13	1.00 ± 0.07	1.22 ± 0.06	
Lymphocytes (10^3/μL)						
Day 25	10.90 ± 0.34	9.74 ± 0.34	9.99 ± 0.38	8.96 ± 0.36**	6.32 ± 0.16**	
Week 14	6.53 ± 0.38	7.43 ± 0.36	7.17 ± 0.67	6.11 ± 0.31	4.88 ± 0.15**	
Monocytes (10^3/μL)						
Day 25	0.27 ± 0.02	0.27 ± 0.02	0.35 ± 0.03*	0.40 ± 0.03**	0.33 ± 0.02*	
Week 14	0.16 ± 0.03	0.16 ± 0.02	0.16 ± 0.03	0.13 ± 0.01	0.12 ± 0.01	
Basophils (10^3/μL)						
Day 25	0.076 ± 0.009	0.066 ± 0.005	0.064 ± 0.008	0.047 ± 0.004**	0.036 ± 0.006**	
Week 14	0.030 ± 0.004	0.037 ± 0.007	0.033 ± 0.004	0.024 ± 0.003	0.027 ± 0.002	
Eosinophils (10^3/μL)						
Day 25	0.04 ± 0.01	0.04 ± 0.00	0.03 ± 0.00*	0.03 ± 0.00**	0.01 ± 0.00**	
Week 14	0.10 ± 0.02	0.07 ± 0.01	0.07 ± 0.01*	0.04 ± 0.01**	0.03 ± 0.00**	
Methemoglobin (g/dL)						
Day 25	0.35 ± 0.03	0.90 ± 0.04**	1.56 ± 0.04**[c]	1.95 ± 0.05**	1.63 ± 0.06**	
Day 88	0.38 ± 0.02	1.37 ± 0.08**	1.95 ± 0.07**	2.29 ± 0.08**	2.03 ± 0.08**	

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Male (continued)						
Hematology (continued)						
n						
Day 25	10	10	9	10	8	0
Day 88	10	10	10	10	9	0
Week 14	10	10	10	10	9	0
Methemoglobin (% hemoglobin)						
Day 25	2.40 ± 0.22	6.70 ± 0.30**	12.44 ± 0.41**	16.60 ± 0.31**	14.75 ± 0.56**	
Day 88	2.44 ± 0.18[d]	10.10 ± 0.55**	15.50 ± 0.48**	18.20 ± 0.53**	17.67 ± 0.71**	
Heinz bodies (% erythrocytes)						
Day 25	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.6**	14.5 ± 1.9**	23.5 ± 2.6**	
Week 14	0.0 ± 0.0	0.5 ± 0.2**	2.8 ± 0.3**	4.1 ± 0.4**	2.9 ± 0.8**	
Clinical Chemistry						
n						
Day 25	10	10	10	10	8	0
Week 14	10	10	10	10	9	0
Urea nitrogen (mg/dL)						
Day 25	13.0 ± 0.3	11.1 ± 0.2*	9.3 ± 0.3**	11.3 ± 0.3	12.0 ± 0.7	
Week 14	14.2 ± 0.3	12.4 ± 0.5	13.8 ± 0.5	13.9 ± 0.4	17.1 ± 0.7*	
Creatinine (mg/dL)						
Day 25	0.47 ± 0.02	0.45 ± 0.02	0.44 ± 0.02	0.40 ± 0.00**	0.34 ± 0.02**	
Week 14	0.60 ± 0.00	0.54 ± 0.02**	0.54 ± 0.02**	0.51 ± 0.02**	0.52 ± 0.01**	
Total protein (g/dL)						
Day 25	6.6 ± 0.1	7.4 ± 0.1**	7.3 ± 0.1**	7.2 ± 0.1**	6.7 ± 0.1	
Week 14	6.8 ± 0.0	7.4 ± 0.1**	7.4 ± 0.1**	7.3 ± 0.1**	7.4 ± 0.1**	
Albumin (g/dL)						
Day 25	4.4 ± 0.0	4.9 ± 0.0**	4.8 ± 0.0**	4.8 ± 0.1**	4.6 ± 0.0	
Week 14	4.6 ± 0.0	5.1 ± 0.0**	5.1 ± 0.1**	5.2 ± 0.0**	5.3 ± 0.0**	
Alanine aminotransferase (IU/L)						
Day 25	45 ± 1	56 ± 2**	88 ± 6**	163 ± 17**	172 ± 19**	
Week 14	72 ± 5	52 ± 4*	62 ± 6	51 ± 4**	58 ± 7	
Alkaline phosphatase (IU/L)						
Day 25	449 ± 9	348 ± 11**	368 ± 9**	353 ± 10**	336 ± 6**	
Week 14	195 ± 5	177 ± 3*	176 ± 3*	188 ± 4	187 ± 4	
Creatine kinase (IU/L)						
Day 25	179 ± 19	120 ± 12	172 ± 21	185 ± 21	409 ± 125	
Week 14	285 ± 55	220 ± 48	168 ± 30	228 ± 53	201 ± 27	
Sorbitol dehydrogenase (IU/L)						
Day 25	18 ± 1	25 ± 1**	40 ± 2**	51 ± 5**	45 ± 6**	
Week 14	22 ± 2	23 ± 2	24 ± 3	18 ± 2	20 ± 2	
Bile acids (µmol/L)						
Day 25	12.1 ± 1.9	13.5 ± 2.5	22.0 ± 4.0	14.6 ± 2.4	21.1 ± 3.1	
Week 14	6.3 ± 1.1	9.1 ± 1.8	11.8 ± 2.3*	13.5 ± 1.6**	18.9 ± 2.5**	

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Female						
Hematology						
n						
Day 25	10	10	10	10	10	0
Day 88	9	10	10	10	10	0
Week 14	10	10	9	10	10	0
Hematocrit (%)						
Day 25	48.8 ± 0.4	44.9 ± 0.3**	43.4 ± 0.6**	40.8 ± 0.5**	37.0 ± 0.5**	
Week 14	45.2 ± 0.5	41.3 ± 0.5**	40.0 ± 0.6**	39.0 ± 0.4**	40.7 ± 0.3**	
Hemoglobin (g/dL)						
Day 25	15.1 ± 0.1	13.3 ± 0.1**	12.8 ± 0.2**	11.7 ± 0.1**	10.8 ± 0.2**	
Week 14	14.8 ± 0.1	12.8 ± 0.1**	12.7 ± 0.1**	12.0 ± 0.2**	12.4 ± 0.1**	
Erythrocytes (10^6/μL)						
Day 25	8.36 ± 0.07	7.42 ± 0.07**	6.90 ± 0.10**	5.93 ± 0.05**	5.15 ± 0.08**	
Week 14	8.16 ± 0.07	6.84 ± 0.08**	6.59 ± 0.10**	6.08 ± 0.10**	5.72 ± 0.06**	
Reticulocytes (10^6/μL)						
Day 25	0.18 ± 0.01	0.55 ± 0.02**	0.62 ± 0.03**	0.99 ± 0.05**	1.07 ± 0.04**	
Week 14	0.26 ± 0.01	0.50 ± 0.03**	0.54 ± 0.02**	0.90 ± 0.02**	1.11 ± 0.04**	
Nucleated erythrocytes/100 leukocytes						
Day 25	0.4 ± 0.2	1.6 ± 0.3**	3.2 ± 0.4**	4.1 ± 0.6**	16.8 ± 1.5**	
Week 14	0.7 ± 0.3	1.4 ± 0.3	2.2 ± 0.3**	3.7 ± 0.4**	5.8 ± 0.7**	
Mean cell volume (fL)						
Day 25	58.4 ± 0.1	60.5 ± 0.2**	62.9 ± 0.3**	68.7 ± 0.4**	71.9 ± 0.6**	
Week 14	55.4 ± 0.2	60.4 ± 0.2**	60.7 ± 0.4**	64.2 ± 0.5**	71.2 ± 0.5**	
Mean cell hemoglobin (pg)						
Day 25	18.0 ± 0.1	17.9 ± 0.1	18.5 ± 0.1**	19.8 ± 0.1**	20.9 ± 0.1**	
Week 14	18.1 ± 0.0	18.7 ± 0.1**	19.3 ± 0.2**	19.8 ± 0.1**	21.7 ± 0.1**	
Mean cell hemoglobin concentration (g/dL)						
Day 25	30.9 ± 0.1	29.5 ± 0.1**	29.4 ± 0.1**	28.8 ± 0.1**	29.0 ± 0.2**	
Week 14	32.7 ± 0.1	31.1 ± 0.1**	31.9 ± 0.2**	30.9 ± 0.2**	30.5 ± 0.1**	
Platelets (10^3/μL)						
Day 25	838.8 ± 28.5	878.2 ± 30.2	894.5 ± 42.5	862.2 ± 31.5	911.2 ± 19.8	
Week 14	751.2 ± 22.6	769.4 ± 17.0	743.0 ± 30.7	656.0 ± 34.1*	607.7 ± 29.2**	
Leukocytes (10^3/μL)						
Day 25	12.94 ± 0.46	12.01 ± 0.67	11.60 ± 0.62*	9.18 ± 0.30**	8.84 ± 0.36**	
Week 14	7.01 ± 0.38	6.90 ± 0.34	7.66 ± 0.53	7.45 ± 0.42	5.07 ± 0.47	
Segmented neutrophils (10^3/μL)						
Day 25	1.14 ± 0.06	1.04 ± 0.07	1.21 ± 0.08	0.87 ± 0.06	1.92 ± 0.10*	
Week 14	1.20 ± 0.05	0.88 ± 0.06**	0.93 ± 0.08**	1.01 ± 0.05*	0.80 ± 0.08**	
Lymphocytes (10^3/μL)						
Day 25	11.40 ± 0.42	10.58 ± 0.61	10.05 ± 0.57	7.92 ± 0.23**	6.37 ± 0.33**	
Week 14	5.55 ± 0.33	5.86 ± 0.29	6.54 ± 0.47	6.29 ± 0.42	4.16 ± 0.38	
Monocytes (10^3/μL)						
Day 25	0.28 ± 0.02	0.25 ± 0.02	0.22 ± 0.02	0.32 ± 0.02	0.47 ± 0.04**	
Week 14	0.16 ± 0.02	0.09 ± 0.01**	0.12 ± 0.01*	0.10 ± 0.01**	0.08 ± 0.01**	
Basophils (10^3/μL)						
Day 25	0.068 ± 0.006	0.088 ± 0.016	0.072 ± 0.010	0.039 ± 0.003**	0.045 ± 0.005**	
Week 14	0.024 ± 0.003	0.024 ± 0.003	0.016 ± 0.002	0.016 ± 0.002	0.010 ± 0.001**	
Eosinophils (10^3/μL)						
Day 25	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.00*	
Week 14	0.09 ± 0.01	0.05 ± 0.00**	0.05 ± 0.01**	0.04 ± 0.00**	0.03 ± 0.00**	
Methemoglobin (g/dL)						
Day 25	0.37 ± 0.02	0.86 ± 0.07**	1.63 ± 0.05**	1.86 ± 0.05**	1.65 ± 0.03**	
Day 88	0.38 ± 0.01	1.49 ± 0.07**	2.20 ± 0.13**	2.49 ± 0.10**	1.75 ± 0.07**	

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of *N,N*-Dimethyl-*p*-toluidine

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Female (continued)						
Hematology (continued)						
n						
Day 25	10	10	10	10	10	0
Day 88	9	10	10	10	10	0
Week 14	10	10	9	10	10	0
Methemoglobin (% hemoglobin)						
Day 25	2.70 ± 0.15	6.40 ± 0.58**	12.80 ± 0.39**	16.00 ± 0.45**	15.50 ± 0.31**	
Day 88	2.88 ± 0.13[e]	11.20 ± 0.44**	17.22 ± 1.18**[d]	19.70 ± 0.62**	16.00 ± 0.42**	
Heinz bodies (% erythrocytes)						
Day 25	0.0 ± 0.0	0.0 ± 0.0	1.5 ± 0.3**	14.4 ± 0.8**	21.2 ± 1.8**	
Week 14	0.0 ± 0.0	0.2 ± 0.0**	4.8 ± 0.7**	6.8 ± 0.6**	16.0 ± 1.8**	
Clinical Chemistry						
n						
Day 25	10	10	10	10	10	0
Week 14	10	10	10	10	10	0
Urea nitrogen (mg/dL)						
Day 25	12.5 ± 0.4	10.5 ± 0.5	9.1 ± 0.5**	10.3 ± 0.5*	11.9 ± 0.3	
Week 14	14.4 ± 0.3	14.4 ± 0.4	15.4 ± 0.5	16.9 ± 0.5**	21.3 ± 1.8**	
Creatinine (mg/dL)						
Day 25	0.46 ± 0.02	0.40 ± 0.00**	0.40 ± 0.00**	0.40 ± 0.00**	0.36 ± 0.02**	
Week 14	0.52 ± 0.01	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00	0.49 ± 0.02	
Total protein (g/dL)						
Day 25	6.1 ± 0.1	6.9 ± 0.1**	7.0 ± 0.1**	6.7 ± 0.1*	6.6 ± 0.1	
Week 14	6.5 ± 0.1	7.0 ± 0.1**	6.9 ± 0.1**	6.9 ± 0.1**	7.0 ± 0.0**	
Albumin (g/dL)						
Day 25	4.3 ± 0.1	4.9 ± 0.0**	5.0 ± 0.1**	4.8 ± 0.0**	4.7 ± 0.1	
Week 14	4.6 ± 0.1	5.1 ± 0.0**	5.1 ± 0.1**	5.2 ± 0.0**	5.3 ± 0.0**	
Alanine aminotransferase (IU/L)						
Day 25	34 ± 1	32 ± 1	43 ± 1**	88 ± 8**	149 ± 9**	
Week 14	53 ± 4	35 ± 1	42 ± 4	61 ± 7	83 ± 18	
Alkaline phosphatase (IU/L)						
Day 25	324 ± 5	245 ± 9**	258 ± 6**	269 ± 10**	276 ± 10*	
Week 14	172 ± 4	151 ± 3	154 ± 3	171 ± 3	215 ± 6*	
Creatine kinase (IU/L)						
Day 25	177 ± 29	170 ± 11	153 ± 14	161 ± 35	185 ± 29	
Week 14	272 ± 39	221 ± 46	234 ± 38	179 ± 22	195 ± 37	
Sorbitol dehydrogenase (IU/L)						
Day 25	13 ± 1	17 ± 1*	27 ± 1**	47 ± 5**	60 ± 4**	
Week 14	16 ± 1	21 ± 1*	25 ± 4*	27 ± 5*	24 ± 5	
Bile acids (µmol/L)						
Day 25	7.2 ± 0.7	11.1 ± 1.6	12.8 ± 1.5**	11.5 ± 1.6**	20.7 ± 3.1**	
Week 14	8.9 ± 1.6	14.1 ± 2.4	13.2 ± 1.0	11.9 ± 1.2	32.2 ± 3.5**	

* Significantly different (P≤0.05) from the vehicle control group by Dunn's or Shirley's test
** P≤0.01
[a] Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.
[b] All 1,000 mg/kg rats died before the end of the study; no data are available for these groups.
[c] n=10
[d] n=9
[e] n=8

TABLE F2
Hematology Data for Rats at 3 Months in the 2-Year Gavage Study of *N,N*-Dimethyl-*p*-toluidine[a]

	Vehicle Control	6 mg/kg	20 mg/kg	60 mg/kg
n	10	10	10	10
Male				
Hematocrit (%)	48.8 ± 0.5	48.4 ± 0.4	46.5 ± 0.3**	42.6 ± 0.3**
Hemoglobin (g/dL)	16.0 ± 0.2	15.6 ± 0.1*	14.7 ± 0.1**	13.2 ± 0.1**
Erythrocytes (10^6/µL)	9.10 ± 0.10	9.02 ± 0.06	8.53 ± 0.04**	7.61 ± 0.06**
Reticulocytes (10^6/µL)	0.25 ± 0.01	0.26 ± 0.01*	0.35 ± 0.01**	0.69 ± 0.02**
Mean cell volume (fL)	53.7 ± 0.2	53.6 ± 0.2	54.5 ± 0.2**	56.0 ± 0.1**
Mean cell hemoglobin (pg)	17.5 ± 0.1	17.3 ± 0.1	17.3 ± 0.1	17.3 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.7 ± 0.2	32.2 ± 0.2	31.6 ± 0.1**	30.9 ± 0.2**
Platelets (10^3/µL)	645.4 ± 27.5	682.6 ± 7.8	721.4 ± 18.4**	722.0 ± 26.0*
Leukocytes (10^3/µL)	9.44 ± 0.49	9.91 ± 0.45	9.99 ± 0.51	9.31 ± 0.58
Segmented neutrophils (10^3/µL)	1.38 ± 0.09	1.42 ± 0.04	1.42 ± 0.09	1.50 ± 0.05
Lymphocytes (10^3/µL)	7.70 ± 0.42	8.10 ± 0.41	8.18 ± 0.41	7.46 ± 0.52
Monocytes (10^3/µL)	0.23 ± 0.02	0.26 ± 0.02	0.24 ± 0.02	0.20 ± 0.02
Basophils (10^3/µL)	0.062 ± 0.007	0.071 ± 0.006	0.079 ± 0.012	0.075 ± 0.009
Eosinophils (10^3/µL)	0.08 ± 0.02	0.07 ± 0.01	0.08 ± 0.01	0.06 ± 0.02
Methemoglobin (g/dL)	0.77 ± 0.04	0.88 ± 0.03*	1.14 ± 0.03**	2.30 ± 0.03**
Methemoglobin (% hemoglobin)	4.70 ± 0.26	5.60 ± 0.22*	7.90 ± 0.18**	17.40 ± 0.22**
Heinz bodies (% erythrocytes)	0.0 ± 0.0	0.1 ± 0.1	0.7 ± 0.2**	3.7 ± 0.3**
Female				
Hematocrit (%)	46.9 ± 0.5	45.8 ± 0.6	44.2 ± 0.6**	41.3 ± 0.6**
Hemoglobin (g/dL)	15.8 ± 0.2	15.1 ± 0.2*	14.4 ± 0.2**	13.2 ± 0.1**
Erythrocytes (10^6/µL)	8.50 ± 0.09	8.31 ± 0.10	7.88 ± 0.08**	6.95 ± 0.09**
Reticulocytes (10^6/µL)	0.24 ± 0.01	0.24 ± 0.01	0.35 ± 0.01**	0.70 ± 0.02**
Mean cell volume (fL)	55.1 ± 0.2	55.1 ± 0.2	56.1 ± 0.3*	59.4 ± 0.2**
Mean cell hemoglobin (pg)	18.6 ± 0.1	18.2 ± 0.1*	18.3 ± 0.1	19.0 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.8 ± 0.2	33.1 ± 0.2*	32.6 ± 0.2**	32.0 ± 0.2**
Platelets (10^3/µL)	597.4 ± 46.6	583.1 ± 46.9	578.8 ± 49.0	719.3 ± 31.9
Leukocytes (10^3/µL)	8.04 ± 0.35	8.65 ± 0.22	8.59 ± 0.56	7.46 ± 0.38
Segmented neutrophils (10^3/µL)	1.40 ± 0.10	1.51 ± 0.11	1.52 ± 0.15	0.95 ± 0.11
Lymphocytes (10^3/µL)	6.29 ± 0.30	6.76 ± 0.26	6.74 ± 0.44	6.24 ± 0.33
Monocytes (10^3/µL)	0.21 ± 0.01	0.24 ± 0.02	0.18 ± 0.02	0.15 ± 0.01*
Basophils (10^3/µL)	0.060 ± 0.007	0.054 ± 0.003	0.065 ± 0.009	0.052 ± 0.006
Eosinophils (10^3/µL)	0.07 ± 0.01	0.09 ± 0.01	0.09 ± 0.02	0.07 ± 0.03
Methemoglobin (g/dL)	0.80 ± 0.03	0.87 ± 0.03	1.21 ± 0.05**	2.26 ± 0.07**
Methemoglobin (% hemoglobin)	5.10 ± 0.23	5.60 ± 0.27	8.40 ± 0.31**	17.10 ± 0.41**
Heinz bodies (% erythrocytes)	0.0 ± 0.0	0.3 ± 0.2*	0.9 ± 0.3**	3.8 ± 0.2**

* Significantly different (P≤0.05) from the vehicle control group by Dunn's or Shirley's test
** Significantly different (P≤0.01) from the vehicle control group by Shirley's test
[a] Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.

TABLE F3
Hematology Data for Mice in the 3-Month Gavage Study of *N,N*-Dimethyl-*p*-toluidine[a]

	Vehicle Control	15 mg/kg	30 mg/kg	60 mg/kg	125 mg/kg	250 mg/kg
Male						
n	10	10	10	10	7	1[b]
Hematocrit (%)	46.6 ± 0.6	43.7 ± 0.5*	45.4 ± 0.6	43.5 ± 0.5**	44.7 ± 0.5	42.5
Hemoglobin (g/dL)	16.4 ± 0.3	15.5 ± 0.2	16.0 ± 0.3	15.0 ± 0.1**	15.3 ± 0.1**	15.7
Erythrocytes (10^6/µL)	10.82 ± 0.18	10.18 ± 0.14*	10.63 ± 0.15	10.14 ± 0.12*	10.27 ± 0.10	9.68
Reticulocytes (10^6/µL)	0.25 ± 0.01	0.24 ± 0.01	0.26 ± 0.01	0.27 ± 0.01	0.28 ± 0.01*	0.37
Mean cell volume (fL)	43.1 ± 0.2	42.9 ± 0.2	42.8 ± 0.1	42.9 ± 0.2	43.5 ± 0.4	43.9
Mean cell hemoglobin (pg)	15.2 ± 0.1	15.2 ± 0.1	15.0 ± 0.2	14.8 ± 0.1*	15.0 ± 0.1	16.2
Mean cell hemoglobin concentration (g/dL)	35.3 ± 0.3	35.4 ± 0.3	35.1 ± 0.4	34.5 ± 0.2	34.4 ± 0.3	36.9
Platelets (10^3/µL)	1,094.7 ± 24.2	1,104.2 ± 59.3	1,158.6 ± 41.0	1,170.3 ± 28.7	1,202.7 ± 25.5*	1,478.0
Leukocytes (10^3/µL)	4.44 ± 0.45	4.05 ± 0.49	4.28 ± 0.47	4.11 ± 0.17	4.79 ± 0.36	8.22
Segmented neutrophils (10^3/µL)	0.93 ± 0.11	0.89 ± 0.08	0.99 ± 0.09	0.81 ± 0.07	1.03 ± 0.10	1.69
Lymphocytes (10^3/µL)	3.32 ± 0.35	3.01 ± 0.41	3.13 ± 0.41	3.17 ± 0.15	3.60 ± 0.29	6.08
Monocytes (10^3/µL)	0.07 ± 0.01	0.08 ± 0.02	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.18
Basophils (10^3/µL)	0.008 ± 0.001	0.006 ± 0.002	0.006 ± 0.002	0.007 ± 0.002	0.004 ± 0.002	0.010
Eosinophils (10^3/µL)	0.11 ± 0.02	0.08 ± 0.01	0.08 ± 0.02	0.07 ± 0.01	0.09 ± 0.02	0.25
Methemoglobin (g/dL)	0.35 ± 0.02	0.36 ± 0.02	0.42 ± 0.02*	0.47 ± 0.02**	0.61 ± 0.03**	0.90
Methemoglobin (% hemoglobin)	2.10 ± 0.10	2.50 ± 0.17	2.80 ± 0.13**	3.10 ± 0.10**	4.00 ± 0.22**	6.00
Heinz bodies (% erythrocytes)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.1**	4.2
Female						
n	10	9	10	10	8	0[c]
Hematocrit (%)	44.9 ± 0.4	43.8 ± 0.6	45.5 ± 0.6	44.9 ± 0.4	46.4 ± 0.7	
Hemoglobin (g/dL)	15.8 ± 0.3	15.5 ± 0.2	16.1 ± 0.2	15.7 ± 0.1	16.1 ± 0.2	
Erythrocytes (10^6/µL)	10.42 ± 0.11	10.13 ± 0.15	10.57 ± 0.14	10.41 ± 0.07	10.64 ± 0.12	
Reticulocytes (10^6/µL)	0.26 ± 0.02	0.26 ± 0.02	0.24 ± 0.02	0.24 ± 0.02	0.31 ± 0.02	
Mean cell volume (fL)	43.1 ± 0.1	43.2 ± 0.1	43.0 ± 0.1	43.1 ± 0.1	43.6 ± 0.2	
Mean cell hemoglobin (pg)	15.1 ± 0.2	15.3 ± 0.1	15.2 ± 0.1	15.1 ± 0.0	15.2 ± 0.0	
Mean cell hemoglobin concentration (g/dL)	35.1 ± 0.4	35.4 ± 0.2	35.3 ± 0.2	35.1 ± 0.1	34.8 ± 0.2*	
Platelets (10^3/µL)	945.3 ± 57.5	918.9 ± 67.8	995.0 ± 40.2	1,044.4 ± 42.2	1,035.1 ± 57.8	
Leukocytes (10^3/µL)	4.87 ± 0.42	4.21 ± 0.45	4.79 ± 0.40	4.36 ± 0.33	4.44 ± 0.19	
Segmented neutrophils (10^3/µL)	0.82 ± 0.14	0.58 ± 0.11	1.11 ± 0.21	0.68 ± 0.07	0.84 ± 0.10	
Lymphocytes (10^3/µL)	3.85 ± 0.28	3.47 ± 0.38	3.51 ± 0.33	3.50 ± 0.26	3.44 ± 0.13	
Monocytes (10^3/µL)	0.09 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.02	
Basophils (10^3/µL)	0.007 ± 0.002	0.004 ± 0.002	0.007 ± 0.002	0.006 ± 0.002	0.010 ± 0.002	
Eosinophils (10^3/µL)	0.10 ± 0.01	0.06 ± 0.02	0.09 ± 0.02	0.10 ± 0.02	0.09 ± 0.01	
Methemoglobin (g/dL)	0.32 ± 0.01	0.34 ± 0.02	0.43 ± 0.02**	0.53 ± 0.02**	0.58 ± 0.03**	
Methemoglobin (% hemoglobin)	2.10 ± 0.10	2.22 ± 0.15	2.60 ± 0.16*	3.40 ± 0.16**	3.88 ± 0.13**	
Heinz bodies (% erythrocytes)	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.2 ± 0.1**	0.5 ± 0.1**	

* Significantly different (P≤0.05) from the vehicle control group by Dunn's or Shirley's test
** P≤0.01
[a] Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.
[b] No standard error was calculated; less than two measurements were available.
[c] All 250 mg/kg female mice died before the end of the study; no data are available for this group.

APPENDIX G
ORGAN WEIGHTS
AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

TABLE G1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Gavage Study of *N,N*-Dimethyl-*p*-toluidine[a]

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Male						
n	10	10	10	10	9	0[b]
Necropsy body wt	327 ± 2	296 ± 5**	289 ± 8**	252 ± 5**	234 ± 9**	
Heart						
Absolute	0.91 ± 0.01	0.90 ± 0.01	0.92 ± 0.02	0.88 ± 0.02	0.85 ± 0.04	
Relative	2.766 ± 0.028	3.038 ± 0.040**	3.192 ± 0.050**	3.482 ± 0.044**	3.634 ± 0.072**	
R. Kidney						
Absolute	1.04 ± 0.02	1.10 ± 0.02	1.17 ± 0.03*	1.08 ± 0.02	1.18 ± 0.06*	
Relative	3.188 ± 0.054	3.721 ± 0.069**	4.042 ± 0.065**	4.298 ± 0.050**	5.021 ± 0.158**	
Liver						
Absolute	11.65 ± 0.15	13.62 ± 0.37*	14.90 ± 0.70**	14.11 ± 0.39**	14.36 ± 0.94**	
Relative	35.606 ± 0.408	46.030 ± 0.754**	51.269 ± 1.082**	56.017 ± 1.057**	60.768 ± 1.748**	
Lung						
Absolute	1.56 ± 0.07	1.49 ± 0.04	1.43 ± 0.05	1.36 ± 0.06*	1.28 ± 0.06**	
Relative	4.769 ± 0.193	5.063 ± 0.144	4.939 ± 0.159	5.396 ± 0.188*	5.519 ± 0.277*	
R. Testis						
Absolute	1.390 ± 0.015	1.416 ± 0.020	1.387 ± 0.030	1.317 ± 0.020	1.247 ± 0.038**	
Relative	4.248 ± 0.047	4.795 ± 0.067**	4.802 ± 0.048**	5.239 ± 0.071**	5.367 ± 0.203**	
Thymus						
Absolute	0.314 ± 0.020	0.263 ± 0.013*	0.261 ± 0.016*	0.233 ± 0.007**	0.200 ± 0.013**	
Relative	0.958 ± 0.062	0.888 ± 0.034	0.896 ± 0.032	0.929 ± 0.038	0.858 ± 0.045	
Female						
n	10	10	10	10	10	0
Necropsy body wt	193 ± 3	183 ± 3*	172 ± 4**	174 ± 3**	175 ± 3**	
Heart						
Absolute	0.63 ± 0.01	0.69 ± 0.02**	0.67 ± 0.01**	0.68 ± 0.01**	0.73 ± 0.01**	
Relative	3.242 ± 0.054	3.753 ± 0.090**	3.878 ± 0.086**	3.907 ± 0.053**	4.198 ± 0.062**	
R. Kidney						
Absolute	0.69 ± 0.01	0.77 ± 0.01**	0.80 ± 0.02**	0.82 ± 0.01**	0.99 ± 0.04**	
Relative	3.560 ± 0.052	4.194 ± 0.042**	4.617 ± 0.066**	4.738 ± 0.066**	5.673 ± 0.157**	
Liver						
Absolute	6.22 ± 0.11	8.61 ± 0.20**	9.11 ± 0.28**	10.61 ± 0.25**	12.61 ± 0.37**	
Relative	32.286 ± 0.427	46.986 ± 0.525**	52.859 ± 0.894**	61.141 ± 0.935**	72.141 ± 1.144**	
Lung						
Absolute	1.21 ± 0.08	1.14 ± 0.05	1.02 ± 0.03*	1.01 ± 0.03**	0.96 ± 0.03**	
Relative	6.268 ± 0.421	6.182 ± 0.234	5.960 ± 0.173	5.813 ± 0.167	5.469 ± 0.130	
Thymus						
Absolute	0.247 ± 0.011	0.219 ± 0.007*	0.210 ± 0.006*	0.226 ± 0.008*	0.190 ± 0.008**	
Relative	1.278 ± 0.048	1.195 ± 0.036	1.224 ± 0.037	1.306 ± 0.048	1.090 ± 0.046*	

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' test
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] All 1,000 mg/kg rats died before the end of the study; no data are available for this group.

TABLE G2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Gavage Study of *N,N*-Dimethyl-*p*-toluidine[a]

	Vehicle Control	15 mg/kg	30 mg/kg	60 mg/kg	125 mg/kg	250 mg/kg
Male						
n	10	10	10	10	7	1[b]
Necropsy body wt	33.6 ± 1.4	35.2 ± 0.5	33.0 ± 1.0	33.1 ± 1.0	29.5 ± 0.4*	26.5
Heart						
Absolute	0.17 ± 0.01	0.18 ± 0.00	0.18 ± 0.01	0.18 ± 0.01	0.16 ± 0.00	0.14
Relative	5.092 ± 0.135	5.016 ± 0.140	5.316 ± 0.157	5.302 ± 0.141	5.333 ± 0.053	5.358
R. Kidney						
Absolute	0.27 ± 0.01	0.30 ± 0.01**	0.28 ± 0.01	0.27 ± 0.01	0.25 ± 0.01	0.23
Relative	7.975 ± 0.244	8.447 ± 0.159	8.474 ± 0.224	8.252 ± 0.138	8.514 ± 0.178	8.717
Liver						
Absolute	1.43 ± 0.07	1.88 ± 0.03**	1.72 ± 0.09**	1.91 ± 0.07**	2.01 ± 0.04**	2.25
Relative	42.520 ± 0.810	53.390 ± 0.817**	51.805 ± 1.324**	57.853 ± 1.401**	68.107 ± 0.984**	84.792
Lung						
Absolute	0.22 ± 0.01	0.25 ± 0.02	0.22 ± 0.01	0.23 ± 0.01	0.28 ± 0.01*	0.25
Relative	6.500 ± 0.301	7.151 ± 0.617	6.692 ± 0.290	6.895 ± 0.343	9.316 ± 0.396**	9.358
R. Testis						
Absolute	0.116 ± 0.002	0.115 ± 0.002	0.116 ± 0.002	0.117 ± 0.002	0.114 ± 0.002	0.105
Relative	3.470 ± 0.079	3.277 ± 0.058	3.540 ± 0.114	3.545 ± 0.092	3.854 ± 0.052**	3.962
Thymus						
Absolute	0.042 ± 0.003	0.044 ± 0.002	0.041 ± 0.002	0.041 ± 0.002	0.038 ± 0.002	0.035
Relative	1.245 ± 0.050	1.247 ± 0.062	1.228 ± 0.051	1.250 ± 0.039	1.297 ± 0.066	1.321
Female						
n	10	10	10	10	8	0[c]
Necropsy body wt	27.7 ± 0.7	29.4 ± 0.5	28.2 ± 0.9	27.8 ± 0.6	26.2 ± 0.3	
Heart						
Absolute	0.13 ± 0.01	0.14 ± 0.00	0.14 ± 0.01	0.14 ± 0.00	0.14 ± 0.00	
Relative	4.799 ± 0.221	4.859 ± 0.161	4.992 ± 0.124	4.928 ± 0.130	5.228 ± 0.062	
R. Kidney						
Absolute	0.16 ± 0.00	0.18 ± 0.01	0.17 ± 0.01	0.18 ± 0.00	0.17 ± 0.00	
Relative	5.930 ± 0.108	5.986 ± 0.170	6.142 ± 0.207	6.311 ± 0.098	6.557 ± 0.138**	
Liver						
Absolute	1.20 ± 0.03	1.47 ± 0.04**	1.51 ± 0.06**	1.60 ± 0.05**	1.71 ± 0.04**	
Relative	43.230 ± 0.576	49.900 ± 1.100**	53.618 ± 1.521**	57.589 ± 1.092**	65.419 ± 1.276**	
Lung						
Absolute	0.23 ± 0.01	0.23 ± 0.01	0.24 ± 0.01	0.25 ± 0.01	0.27 ± 0.01**	
Relative	8.166 ± 0.464	7.755 ± 0.428	8.467 ± 0.561	8.832 ± 0.325	10.484 ± 0.314**	
Thymus						
Absolute	0.053 ± 0.002	0.053 ± 0.002	0.049 ± 0.002	0.047 ± 0.003	0.039 ± 0.002**	
Relative	1.918 ± 0.068	1.782 ± 0.073	1.744 ± 0.078	1.698 ± 0.106	1.501 ± 0.070**	

* Significantly different (P≤0.05) from the vehicle control group by Williams' or Dunnett's test
** P≤0.01
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] No standard error was calculated and statistical analysis was not performed; less than two measurements were available.
[c] All 250 mg/kg female mice died before the end of the study; no data are available for this group.

APPENDIX H
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE H1
Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Gavage Study of *N,N*-Dimethyl-*p*-toluidine[a]

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	327 ± 2	296 ± 5**	289 ± 8**	252 ± 5**
L. Cauda epididymis	0.1440 ± 0.0033	0.1259 ± 0.0046*	0.1392 ± 0.0050	0.1178 ± 0.0049**
L. Epididymis	0.4241 ± 0.0049	0.3955 ± 0.0116	0.4170 ± 0.0102	0.3740 ± 0.0101**
L. Testis	1.4584 ± 0.0189	1.5586 ± 0.0776	1.4644 ± 0.0305	1.4121 ± 0.0487
Spermatid measurements				
Spermatid heads (10^6/testis)	172.00 ± 4.11	172.00 ± 5.31	180.00 ± 5.53	167.13 ± 6.96
Spermatid heads (10^6/g testis)	129.6 ± 3.0	124.4 ± 6.7	135.0 ± 4.6	131.5 ± 4.2
Epididymal spermatozoal measurements				
Sperm motility (%)	82.2 ± 1.2	81.2 ± 1.2	82.8 ± 0.9	81.3 ± 0.8
Sperm (10^6/cauda epididymis)	98.63 ± 9.33	81.88 ± 4.89	82.13 ± 3.57	73.13 ± 5.81
Sperm (10^6/g cauda epididymis)	682 ± 58	659 ± 47	598 ± 33	617 ± 32

* Significantly different (P≤0.05) from the vehicle control group by Dunnett's test
** Significantly different (P≤0.01) from the vehicle control group by Dunnett's test (cauda epididymis weight) or Williams' test (body and epididymis weights)
[a] Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (testis weight) or Dunn's test (spermatid and epididymal spermatozoal measurements).

TABLE H2
Estrous Cycle Characterization for Female Rats in the 3-Month Gavage Study of *N,N*-Dimethyl-*p*-toluidine[a]

	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	193 ± 3	183 ± 3*	172 ± 4**	174 ± 3**
Proportion of regular cycling females[b]	9/10	10/10	8/9	4/4
Estrous cycle length (days)	4.95 ± 0.17	5.10 ± 0.15	5.56 ± 0.32[c]	5.38 ± 0.47[d]
Estrous stages[e] (% of cycle)				
Diestrus	51.7	55.8	59.2	60.0
Proestrus	12.5	13.3	12.5	7.5
Estrus	25.0	23.3	17.5	13.3
Metestrus	4.2	6.7	8.3	7.5
Not clear or no cells observed	6.7	0.8	2.5	11.7

* Significantly different (P≤0.05) from the vehicle control group by Williams' test
** P≤0.01
[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunn's test (estrous cycle length).
[b] Number of females with a regular cycle/number of females cycling
[c] Estrous cycle was longer than 12 days or unclear in 2 of 10 animals.
[d] Estrous cycle was longer than 12 days or unclear in 6 of 10 animals.
[e] Evidence shows that females exposed to 125 mg/kg differ significantly (Wilkes' Criterion, P≤0.05) from the vehicle control females in the relative length of time spent in the estrous stages. Tests for equality of transition probability matrices among all groups and between the vehicle control group and each dosed group indicated that females in the 250 mg/kg dose group spent significantly (P<0.001) more time in extended diestrus than females in the vehicle control group.

Dose (mg/kg)																					
0									E	D	D	D	P	E	E	M	D	D	P	E	
0									E	D	D	D	P	E	D	D	D	P	E	D	
0									E	D	D	D	P	E	D	D	D	P	E	P	
0							D	P	E	D	D	D	IC	E	D	D	D	D			
0								E	E	D	D	D	D	E	D	D	D	D	IC		
0									E	D	D	D	P	E	E	D	D	P	IC	E	
0								P	E	D	D	D	D	E	D	D	D	P	E		
0					D	D	D	P	E	M	D	D	IC	E	M	IC					
0							D	IC	P	E	D	D	D	E	E	M	D	IC			
0								D	P	E	D	D	D	E	E	M	D	D	IC		
62.5										E	M	D	D	E	E	M	D	D	D	E	E
62.5									E	M	D	D	P	E	D	D	D	D	P	E	
62.5					M	D	D	D	E	M	D	D	P	E	E	D					
62.5							D	P	E	D	D	D	P	E	D	D	D	E			
62.5						D	D	P	E	D	D	D	D	E	D	D	D				
62.5							E	M	D	D	D	D	P	E	D	D	D	P			
62.5								P	E	M	D	D	P	E	D	D	D	P	E		
62.5									E	M	D	D	P	E	D	D	D	P	E	D	
62.5								D	P	E	D	D	D	E	D	D	D	D	E		
62.5		D	D	P	E	D	D	D	P	IC	D	D	D								
125								E	M	D	D	D	D	E	E	D	D	D	P		
125		D	P	E	M	D	D	D	P	IC	M	D	D								
125							D	D	E	D	D	D	P	E	D	D	D	P			
125									D	E	M	D	D	E	D	D	D	E	D	D	
125							D	P	E	M	D	D	P	E	M	D	D	P			
125		IC	D	D	D	D	D	D	D	D	P	E	D								
125									E	M	D	D	P	E	D	D	D	D	P	E	
125				D	D	D	D	E	M	D	D	IC	P	E	D						
125					D	D	P	E	M	D	D	D	P	E	D	D					
125			P	E	M	D	D	D	D	D	D	D	D	E							
250				D	D	D	P	E	M	D	D	IC	D	E	D						
250					D	D	P	E	M	D	D	D	IC	E	E	M					
250		D	D	D	D	D	D	D	IC	D	D	IC	D								
250				D	D	D	D	P	E	E	D	D	D	E	M						
250									E	D	D	D	P	E	D	D	D	D	P	IC	
250		D	D	D	D	D	D	D	D	IC	D	D	IC								
250		D	P	E	M	D	D	D	IC	D	P	IC	D								
250		M	D	D	D	P	M	D	IC	IC	D	D	IC								
250									E	M	D	D	D	E	E	M	D	D	IC	E	
250		D	D	IC	D	E	D	D	D	D	D	D	P								

FIGURE H1
Vaginal Cytology Plots for Female Rats in the 3-Month Gavage Study of *N,N*-Dimethyl-*p*-toluidine
(D = diestrus, P = proestrus, E = estrus, M = metestrus, IC = insufficient number of cells to determine stage)

TABLE H3
Results of Vaginal Cytology Study Using the Transition Matrix Approach in Female Rats Administered *N,N*-Dimethyl-*p*-toluidine by Gavage for 3 Months

Stage	Comparison[a]	P-Value	Trend[b]
Overall Tests	Overall	<0.001	
Overall Tests	Low vs. Controls	0.32	N
Overall Tests	Mid vs. Controls	<0.001	N
Overall Tests	High vs. Controls	<0.001	N
Extended Estrus	Overall	0.441	
Extended Estrus	Low vs. Controls	0.509	N
Extended Estrus	Mid vs. Controls	0.136	N
Extended Estrus	High vs. Controls	0.776	N
Extended Diestrus	Overall	<0.001	
Extended Diestrus	Low vs. Controls	0.896	N
Extended Diestrus	Mid vs. Controls	0.002	N
Extended Diestrus	High vs. Controls	<0.001	
Extended Metestrus	Overall	1	
Extended Metestrus	Low vs. Controls	1	
Extended Metestrus	Mid vs. Controls	1	
Extended Metestrus	High vs. Controls	1	
Extended Proestrus	Overall	1	
Extended Proestrus	Low vs. Controls	1	
Extended Proestrus	Mid vs. Controls	1	
Extended Proestrus	High vs. Controls	1	
Skipped Estrus	Overall	1	
Skipped Estrus	Low vs. Controls	1	
Skipped Estrus	Mid vs. Controls	1	
Skipped Estrus	High vs. Controls	0.914	
Skipped Diestrus	Overall	0.379	
Skipped Diestrus	Low vs. Controls	0.291	N
Skipped Diestrus	Mid vs. Controls	0.298	N
Skipped Diestrus	High vs. Controls	0.346	N
Summary of Significant Groups			
Overall Tests	Mid vs. Controls	<0.001	N
Overal Tests	High vs. Controls	<0.001	N
Extended Diestrus	Mid vs. Controls	0.002	N
Extended Diestrus	High vs. Controls	<0.001	

[a] Controls = Vehicle Control, Low = 62.5 mg/kg, Mid = 125 mg/kg, High = 250 mg/kg

[b] N means that the treated group had a lower probability of transitioning to the relevant abnormal state (extended estrus, extended metestrus, extended proestrus, skipped estrus, or skipped diestrus) than did the vehicle control group.

TABLE H4
Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Gavage Study of *N,N*-Dimethyl-*p*-toluidine[a]

	Vehicle Control	15 mg/kg	30 mg/kg	60 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	33.6 ± 1.4	35.2 ± 0.5	33.0 ± 1.0	33.1 ± 1.0
L. Cauda epididymis	0.0160 ± 0.0007	0.0163 ± 0.0003	0.0152 ± 0.0004	0.0152 ± 0.0003
L. Epididymis	0.0431 ± 0.0010	0.0448 ± 0.0008	0.0423 ± 0.0006	0.0441 ± 0.0009
L. Testis	0.1084 ± 0.0017	0.1103 ± 0.0019	0.1124 ± 0.0012	0.1112 ± 0.0021
Spermatid measurements				
Spermatid heads (10^6/testis)	23.19 ± 0.33	22.17 ± 0.85	21.94 ± 1.10	21.80 ± 0.95
Spermatid heads (10^6/g testis)	226.2 ± 4.1	214.0 ± 6.9	204.4 ± 10.0	209.4 ± 8.3
Epididymal spermatozoal measurements				
Sperm motility (%)	82.20 ± 0.87	81.20 ± 0.83	81.20 ± 1.05	82.70 ± 0.84
Sperm (10^6/cauda epididymis)	16.50 ± 1.65	16.98 ± 1.39	16.88 ± 1.07	11.96 ± 0.79
Sperm (10^6/g cauda epididymis)	1,026 ± 75	1,042 ± 79	1,121 ± 78	788 ± 53

[a] Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body and tissue weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).

TABLE H5
Estrous Cycle Characterization for Female Mice in the 3-Month Gavage Study of *N,N*-Dimethyl-*p*-toluidine[a]

	Vehicle Control	15 mg/kg	30 mg/kg	60 mg/kg
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	27.7 ± 0.7	29.4 ± 0.5	28.2 ± 0.9	27.8 ± 0.6
Proportion of regular cycling females[b]	6/7	8/9	8/9	9/10
Estrous cycle length (days)	4.21 ± 0.15[c]	4.31 ± 0.13[d]	4.11 ± 0.18[e]	4.17 ± 0.14
Estrous stages (% of cycle)				
Diestrus	37.5	41.7	41.7	30.8
Proestrus	0.0	0.0	0.0	0.0
Estrus	36.7	35.8	39.2	45.0
Metestrus	18.3	19.2	19.2	23.3
Not clear or no cells observed	7.5	3.3	0.0	0.8

[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body weight) or Dunn's test (estrous cycle length). By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages. Tests for equality of transition probability matrices among all groups and between the vehicle control group and each dosed group indicated dosed females did not spend significantly more time in extended estrus or diestrus than did the vehicle control females.
[b] Number of females with a regular cycle/number of females cycling
[c] Estrous cycle was longer than 12 days or unclear in 3 of 10 animals.
[d] Estrous cycle was longer than 12 days or unclear in 2 of 10 animals.
[e] Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.

APPENDIX I
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

N,N-Dimethyl-*p*-toluidine

N,N-Dimethyl-*p*-toluidine was obtained from Alfa Aesar, a Johnson Matthey Company (Ward Hill, MA), in two lots (H3124A and J7601A). Lot H3124A was used in the 3-month studies. The remainder of lot H3124A was combined with lot J7601A to make lot 050404 which was used in the 2-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory at Battelle's Chemistry Support Services (Columbus, Ohio) and by the study laboratory at Battelle Columbus Operations (Columbus, OH). Karl Fischer titration and elemental analyses were performed by Galbraith Laboratories, Inc. (Knoxville, TN), for the 3-month studies and by Prevalere Life Sciences, Inc. (Whitesboro, NY) for the 2-year studies. Reports on analyses performed in support of the *N,N*-dimethyl-*p*-toluidine studies are on file at the National Institute of Environmental Health Sciences.

All lots of the chemical, a pale-yellow liquid, were identified as *N,N*-dimethyl-*p*-toluidine by the analytical chemistry laboratory and the study laboratory using infrared (IR) spectroscopy. Identity confirmation of lot H3124A and combined lot 050404 was conducted by the analytical chemistry laboratory using proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopy. All spectra were consistent with the literature spectra (*Aldrich*, 1997; *Sadtler*, 2004; *Sadtler-Aldrich*, 2004; *Sigma-Aldrich*, 2004; SDBS, 2004) and the structure of *N,N*-dimethyl-*p*-toluidine. Representative IR and proton NMR spectra are presented in Figures I1 and I2.

For lot H3124A and combined lot 050404, Karl Fischer titration was used to determine the water content, elemental analysis was used to determine carbon, hydrogen, and nitrogen content, and a boiling point determination was performed (lot H3124A only). The purity of all lots was determined by the analytical chemistry laboratory using gas chromatography (GC) by system A. Differential scanning calorimetry (DSC) was also used to determine the purity of lot H3124A. The DSC method included a PerkinElmer DSC-7 instrument (PerkinElmer, Waltham, MA), scanning from –55° to 10° C (–55° to –20° C for the third replicate) at a scanning rate of 1° C per minute under a nitrogen atmosphere; the van't Hoff equation was used for purity calculations.

(A) The GC system included a gas chromatography instrument (Hewlett-Packard or Agilent, Palo Alto, CA) with flame ionization detection (FID), a RTX-5, 30 m × 0.32 mm, 0.25-µm film thickness (Restek, Bellefonte, PA) column, helium carrier gas at a flow rate of approximately 1.4 mL/minute, an oven temperature program of 50° C, held 4 minutes, then 15° C/minute to 180° C, then 20° C/minute to 250° C, held 3 minutes.

For lot H3124A, Karl Fischer titration indicated approximately 0.22% water. Boiling point determination and elemental analyses results for carbon, hydrogen, and nitrogen were consistent with theoretical values. GC/FID indicated one major peak and two impurities (0.1% and 0.2%) with peak areas greater than or equal to 0.1% of the major peak area. DSC indicated a purity of 99.8%. The overall purity of lot H3124A was determined to be greater than 99%.

For lot J7601A, GC/FID indicated one major peak and three impurities (0.1%, 0.1%, and 0.2%) with peak areas greater than or equal to 0.1% of the total peak area. The overall purity of lot J7601A was determined to be greater than 99% and was sufficiently similar to lot H3124A to allow the two lots to be combined.

For combined lot 050404, Karl Fischer titration indicated approximately 0.2% water; elemental analyses for carbon, hydrogen, and nitrogen were consistent with theoretical values. GC/FID indicated one major peak and four impurities (0.2%, 0.1%, 0.2%, and 0.1%) with peak areas greater than or equal to 0.1% of the total peak area. The overall purity of combined lot 050404 was determined to be greater than 99%.

To ensure stability, the bulk chemical was stored in amber glass containers sealed with Teflon®-lined lids at room temperature. Periodic reanalyses of the bulk chemical using GC/FID by a system similar to system A were performed by the study laboratory at the beginning, middle, and end of the 3-month studies and at least every 6 months during the 2-year studies; no degradation of the bulk chemical was observed.

Corn Oil

USP-grade corn oil was obtained in multiple lots from Spectrum Chemicals and Laboratory Products (Gardena, CA) and was used as the vehicle in the 3-month and 2-year studies. Periodic analyses of the corn oil vehicle performed by the study laboratory using potentiometric titration demonstrated peroxide concentrations below the acceptable limit of 3 mEq/kg.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

Dose formulations were prepared by adding the appropriate amount of *N,N*-dimethyl-*p*-toluidine to corn oil to achieve the desired concentration (Table I1). After October 21, 2003, during the 3-month studies, the 400 mg/mL dose formulation was discontinued due to the termination of all animals in the 1,000 mg/kg group. Dose formulations were prepared three times for the 3-month studies and approximately monthly for the 2-year studies.

The 400 mg/mL dose formulation was prepared and observed to be a true solution, therefore, no homogeneity or gavageability studies were performed. Stability studies of a 1.0 mg/mL formulation in corn oil were performed by the analytical chemistry laboratory using GC/FID by system A. Stability was confirmed for up to 44 days for formulations stored in amber glass containers sealed with Teflon®-lined lids, protected from light, at up to room temperature and for at least 3 hours under simulated animal room conditions.

Periodic analyses of the dose formulations were conducted by the study laboratory using GC/FID by a system similar to system A, with the exception of the following oven temperature program, which provided greater separation (50° C, held 4 minutes, then 15° C/minute to 180° C, then 20° C/minute to 260° C, held 5 minutes).

During the 3-month studies, the dose formulations were analyzed at the beginning, midpoint, and end of the studies; animal room samples of these dose formulations were also analyzed (Table I2). Of the dose formulations analyzed and used, all 13 for rats and all 15 for mice were within 10% of the target concentrations; all 13 of the animal room samples for rats and all 15 for mice were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed at least every 3 months; animal room samples were also analyzed (Table I3). Of the dose formulations analyzed and used, all 30 for rats and all 30 for mice were within 10% of the target concentrations; all 12 of the animal room samples for rats and all 12 for mice were within 10% of the target concentrations.

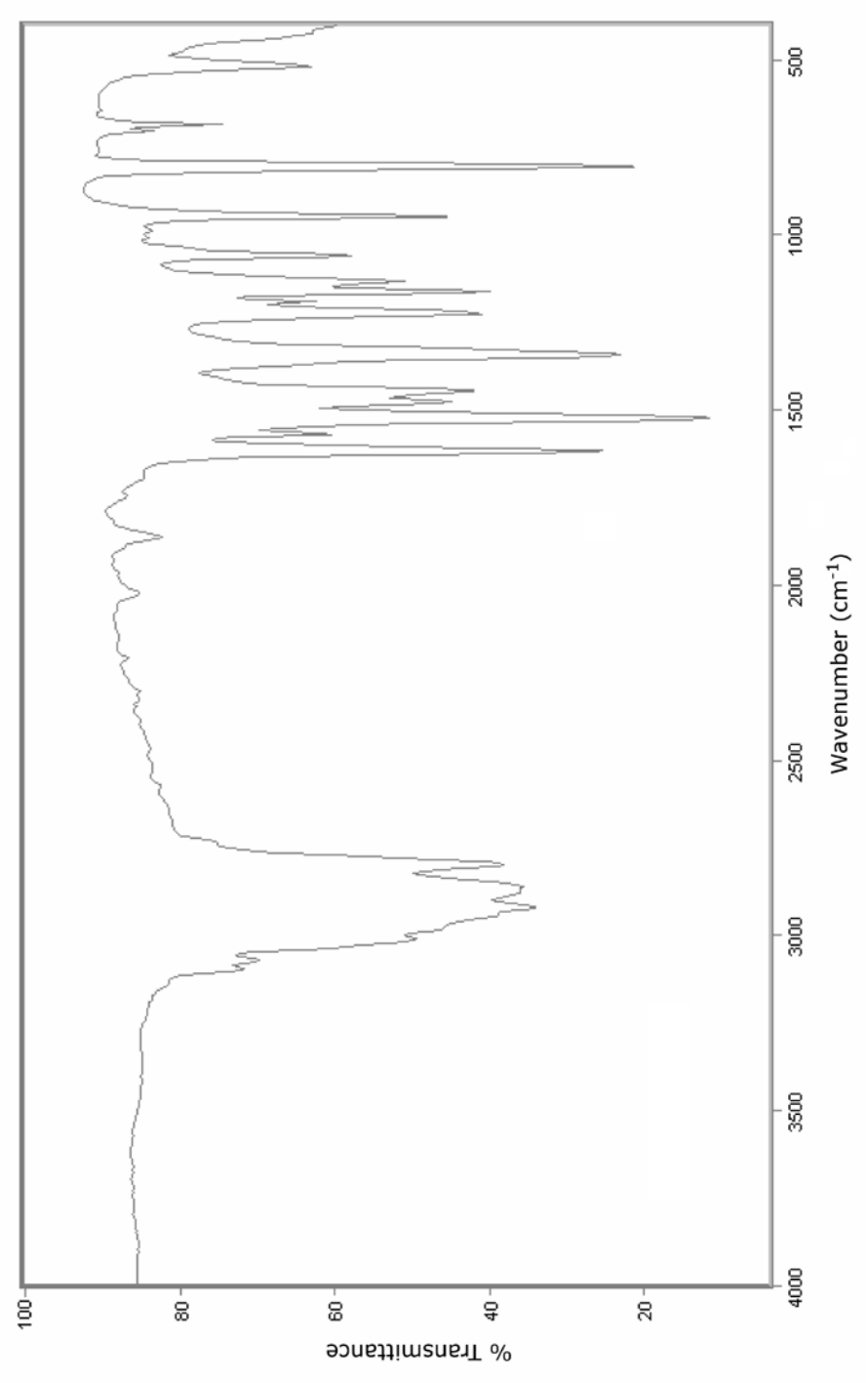

FIGURE I1
Infrared Absorption Spectrum of *N,N*-Dimethyl-*p*-toluidine

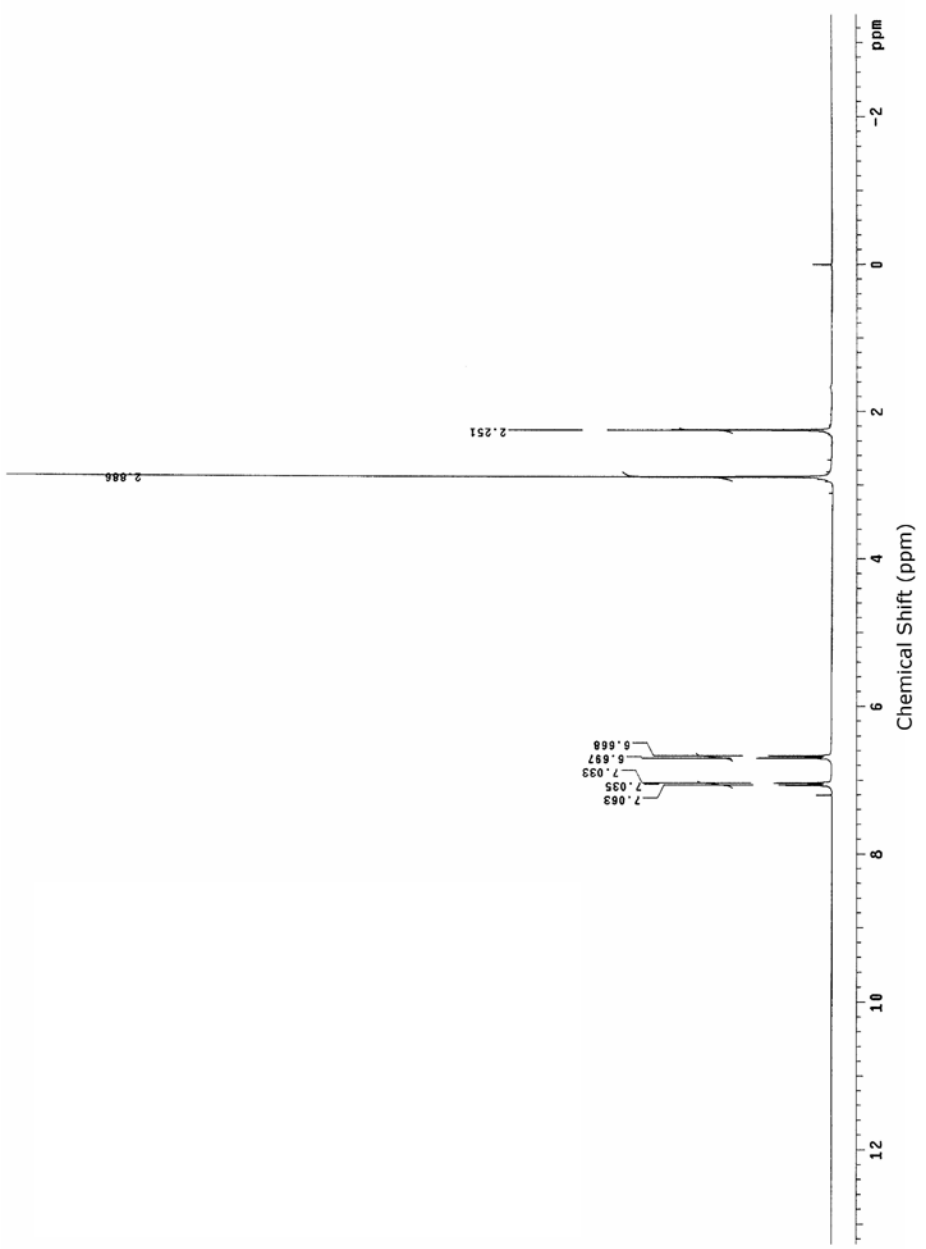

FIGURE I2
Proton Nuclear Magnetic Resonance Spectrum of *N,N*-Dimethyl-*p*-toluidine

TABLE I1
Preparation and Storage of Dose Formulations in the Gavage Studies of *N,N*-Dimethyl-*p*-toluidine

3-Month Studies	2-Year Studies
Preparation	
Dose formulations were prepared by adding approximately half the required volume of corn oil to a calibrated glass mixing bottle. The appropriate amount of *N,N*-dimethyl-*p*-toluidine was measured using a volumetric pipette or a graduated cylinder and transferred to the mixing container with rinsing using corn oil (graduated cylinder only). The bottle was capped and shaken vigorously by hand, the solution was allowed to settle, then the bottle was filled to volume with corn oil, capped and shaken vigorously again by hand, then stirred on a stirplate for approximately 15 minutes. After October 21, 2003, the 400 mg/mL dose formulation was discontinued due to the termination of all animals in this group. Dose formulations were prepared three times.	Same as 3-month studies, except formulations were stirred on a stirplate for approximately 5 minutes. Dose formulations were prepared monthly or as needed.
Chemical Lot Number	
H3124A	050404
Maximum Storage Time	
42 days	42 days
Storage Conditions	
Stored in amber glass bottles sealed with Teflon®-lined lids, protected from light, at room temperature	Stored in amber glass bottles sealed with Teflon®-lined lids, protected from light, at room temperature
Study Laboratory	
Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)

TABLE I2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Gavage Studies of *N,N*-Dimethyl-*p*-toluidine

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
October 7, 2003	October 13-14, 2003	25	23.82	–5
		50	49.21	–2
		100	99.24	–1
		200	200.4	0
		400	397.5	–1
	November 21-22, 2003[b]	25	23.12	–8
		50	47.78	–4
		100	96.31	–4
		200	190.1	–5
		400	388.6	–3
November 4, 2003	November 6-7, 2003	25	23.93	–4
		50	48.59	–3
		100	96.28	–4
		200	189.9	–5
	December 23-24, 2003[b]	25	23.55	–6
		50	48.61	–3
		100	93.77	–6
		200	182.0	–9
December 30, 2003	January 5-6, 2004	25	24.79	–1
		50	50.18	0
		100	96.66	–3
		200	198.3	–1
	February 6-7, 2004[b]	25	23.60	–6
		50	48.71	–3
		100	95.24	–5
		200	191.9	–4
Mice				
October 7, 2003	October 13-14, 2003	3	3.023	+1
		6	5.975	0
		12	11.75	–2
		25	23.82	–5
		50	49.21	–2
	November 21-22, 2003[b]	3	2.831	–6
		6	5.657	–6
		12	11.41	–5
		25	23.93	–4
		50	47.70	–5

TABLE I2
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Gavage Studies of *N,N*-Dimethyl-*p*-toluidine

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice (continued)				
November 4, 2003	November 6-7, 2003	3	2.900	–3
		6	5.768	–4
		12	11.78	–2
		25	23.93	–4
		50	48.59	–3
	December 23-24, 2003[b]	3	2.833	–6
		6	5.564	–7
		12	11.50	–4
		25	23.83	–5
		50	47.97	–4
December 30, 2003	January 5-6, 2004	3	2.951	–2
		6	6.044	+1
		12	12.22	+2
		25	24.79	–1
		50	50.18	0
	February 6-9, 2004[b]	3	2.961	–1
		6	5.925 ± 0.031[c]	–1
		12	11.91	–1
		25	23.99	–4
		50	48.82 ± 0.20[c]	–2

[a] Results of duplicate analyses. Dosing volume for rats=2.5 mL/kg: 25 mg/mL=62.5 mg/kg; 50 mg/mL=125 mg/kg; 100 mg/mL=250 mg/kg; 200 mg/mL=500 mg/kg; 400 mg/mL=1,000 mg/kg. Dosing for mice=5 mL/kg: 3 mg/mL=15 mg/kg; 6 mg/mL=30 mg/kg; 12 mg/mL=60 mg/kg; 25 mg/mL=125 mg/kg; 50 mg/mL=250 mg/kg

[b] Animal room samples

[c] Mean ± standard deviation; results presented are an average of three runs. The initial duplicate set did not pass the acceptance criterion, and another duplicate set was analyzed. One of the original set was eliminated as an outlier based on the Q-test.

TABLE I3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of *N,N*-Dimethyl-*p*-toluidine

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Rats				
October 6, 2004	October 12, 2004	2.4	2.282	–5
		8	7.644	–5
		24	22.78	–5
	November 16-17, 2004[b]	2.4	2.345	–2
		8	7.909	–1
		24	23.91	0
December 22, 2004	December 22-23, 2004	2.4	2.297	–4
		8	7.814	–2
		24	22.90	–5
March 8, 2005	March 9-10, 2005	2.4	2.393	0
		8	7.861	–2
		24	23.54	–2
May 25, 2005	May 26-27, 2005	2.4	2.261	–6
		8	7.692	–4
		24	23.27	–3
	June 29-30, 2005[b]	2.4	2.434	+1
		8	8.100	+1
		24	24.14	+1
August 9, 2005	August 11-12, 2005	2.4	2.374	–1
		8	7.937	–1
		24	24.22	+1
October 25, 2005`	October 26-27, 2005	2.4	2.335	–3
		8	8.076	+1
		24	23.65	–2
January 11, 2006	January 12-13, 2006	2.4	2.353	–2
		8	7.587	–5
		24	22.94	–4
	February 21-22, 2006[b]	2.4	2.393	0
		8	8.030	0
		24	23.28	–3
March 28, 2006	March 31-April 1, 2006	2.4	2.500	+4
		8	8.184	+2
		24	24.14	+1
June 13, 2006	June 15-16, 2006	2.4	2.399	0
		8	8.045	+1
		24	23.21	–3
August 29, 2006	August 30-31, 2006	2.4	2.397	0
		8	8.176	+2
		24	23.19	–3
	October 12-13, 2006[b]	2.4	2.291	–5
		8	7.809	–2
		24	21.96	–9

TABLE I3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of *N,N*-Dimethyl-*p*-toluidine

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice				
October 6, 2004	October 12, 2004	1.2	1.131	–6
		4	3.715	–7
		12	11.28	–6
	November 16-17, 2004[b]	1.2	1.161	–3
		4	3.759	–6
		12	11.34	–6
December 22, 2004	December 22-23, 2004	1.2	1.139	–5
		4	3.713	–7
		12	11.43	–5
March 8, 2005	March 9-10, 2005	1.2	1.185	–1
		4	3.710	–7
		12	11.48	–4
May 25, 2005	May 26-27, 2005	1.2	1.159	–3
		4	3.773	–6
		12	11.64	–3
	June 29-30, 2005[b]	1.2	1.173	–2
		4	3.902	–2
		12	11.52	–4
August 9, 2005	August 11-12, 2005	1.2	1.189	–1
		4	3.859	–4
		12	11.64	–3
October 25, 2005	October 26-27, 2005	1.2	1.177	–2
		4	3.795	–5
		12	11.68	–3
January 11, 2006	January 12-13, 2006	1.2	1.164	–3
		4	3.837	–4
		12	11.50	–4
	February 21-22, 2006[b]	1.2	1.170	–3
		4	3.879	–3
		12	11.78	–2
March 28, 2006	March 31-April 1, 2006	1.2	1.211	+1
		4	3.997	0
		12	12.27	+2
June 13, 2006	June 15-16, 2006	1.2	1.176	–2
		4	3.796	–5
		12	11.76	–2

TABLE I3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of *N,N*-Dimethyl-*p*-toluidine

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice (continued)				
August 29, 2006	August 30-31, 2006	1.2	1.184	–1
		4	3.833	–4
		12	11.81	–2
	October 12-13, 2006[b]	1.2	1.177	–2
		4	3.689	–8
		12	11.29	–6

[a] Results of duplicate analyses. Dosing volume for rats=2.5 mL/kg: 2.4 mg/mL=6 mg/kg; 8 mg/mL=20 mg/kg; 24 mg/mL=60 mg/kg. Dosing volume for mice=5 mL/kg: 1.2 mg/mL=6 mg/kg; 4 mg/mL=20 mg/kg; 12 mg/mL=60 mg/kg.
[b] Animal room samples

APPENDIX J
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NTP-2000 RAT AND MOUSE RATION

TABLE J1
Ingredients of NTP-2000 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground hard winter wheat	22.26
Ground #2 yellow shelled corn	22.18
Wheat middlings	15.0
Oat hulls	8.5
Alfalfa meal (dehydrated, 17% protein)	7.5
Purified cellulose	5.5
Soybean meal (49% protein)	5.0
Fish meal (60% protein)	4.0
Corn oil (without preservatives)	3.0
Soy oil (without preservatives)	3.0
Dried brewer's yeast	1.0
Calcium carbonate (USP)	0.9
Vitamin premix[a]	0.5
Mineral premix[b]	0.5
Calcium phosphate, dibasic (USP)	0.4
Sodium chloride	0.3
Choline chloride (70% choline)	0.26
Methionine	0.2

[a] Wheat middlings as carrier
[b] Calcium carbonate as carrier

TABLE J2
Vitamins and Minerals in NTP-2000 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	4,000 IU	Stabilized vitamin A palmitate or acetate
D	1,000 IU	D-activated animal sterol
K	1.0 mg	Menadione sodium bisulfite complex
α-Tocopheryl acetate	100 IU	
Niacin	23 mg	
Folic acid	1.1 mg	
d-Pantothenic acid	10 mg	*d*-Calcium pantothenate
Riboflavin	3.3 mg	
Thiamine	4 mg	Thiamine mononitrate
B_{12}	52 µg	
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	*d*-Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

[a] Per kg of finished product

TABLE J3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.6 ± 0.67	13.5 – 16.3	25
Crude fat (% by weight)	8.3 ± 0.37	7.6 – 9.3	25
Crude fiber (% by weight)	9.2 ± 0.45	8.4 – 10.0	25
Ash (% by weight)	5.0 ± 0.24	4.6 – 5.4	25
Amino Acids (% of total diet)			
Arginine	0.778 ± 0.068	0.670 – 0.970	21
Cystine	0.220 ± 0.025	0.150 – 0.250	21
Glycine	0.701 ± 0.042	0.620 – 0.800	21
Histidine	0.354 ± 0.079	0.270 – 0.680	21
Isoleucine	0.544 ± 0.045	0.430 – 0.660	21
Leucine	1.092 ± 0.068	0.960 – 1.240	21
Lysine	0.704 ± 0.112	0.310 – 0.840	21
Methionine	0.409 ± 0.047	0.260 – 0.490	21
Phenylalanine	0.626 ± 0.040	0.540 – 0.720	21
Threonine	0.503 ± 0.043	0.430 – 0.610	21
Tryptophan	0.148 ± 0.027	0.110 – 0.200	21
Tyrosine	0.397 ± 0.058	0.280 – 0.540	21
Valine	0.666 ± 0.044	0.550 – 0.730	21
Essential Fatty Acids (% of total diet)			
Linoleic	3.92 ± 0.227	3.49 – 4.54	21
Linolenic	0.30 ± 0.030	0.21 – 0.35	21
Vitamins			
Vitamin A (IU/kg)	3,927 ± 772	2,340 – 5,590	25
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	80.1 ± 22.48	27.0 – 124.0	21
Thiamine (ppm)[b]	7.8 ± 1.17	6.3 – 10.5	25
Riboflavin (ppm)	7.1 ± 1.91	4.20 – 11.20	21
Niacin (ppm)	78.6 ± 9.16	66.4 – 98.2	21
Pantothenic acid (ppm)	27.1 ± 12.89	17.4 – 81.0	21
Pyridoxine (ppm)[b]	9.47 ± 2.01	6.4 – 13.7	21
Folic acid (ppm)	1.63 ± 0.49	1.15 – 3.27	21
Biotin (ppm)	0.319 ± 0.10	0.200 – 0.704	21
Vitamin B_{12} (ppb)	53.8 ± 40.6	18.3 – 174.0	21
Choline (ppm)[b]	2,885 ± 459	1,820 – 3,790	21
Minerals			
Calcium (%)	0.979 ± 0.049	0.895 – 1.080	25
Phosphorus (%)	0.570 ± 0.030	0.515 – 0.623	25
Potassium (%)	0.663 ± 0.027	0.626 – 0.732	21
Chloride (%)	0.387 ± 0.039	0.300 – 0.474	21
Sodium (%)	0.190 ± 0.016	0.160 – 0.222	21
Magnesium (%)	0.216 ± 0.063	0.185 – 0.490	21
Sulfur (%)	0.170 ± 0.029	0.116 – 0.209	14
Iron (ppm)	185 ± 40.1	135 – 311	21
Manganese (ppm)	51.6 ± 10.49	21.0 – 73.1	21
Zinc (ppm)	53.6 ± 8.62	43.3 – 78.5	21
Copper (ppm)	7.07 ± 2.611	3.21 – 16.30	21
Iodine (ppm)	0.497 ± 0.209	0.158 – 0.972	21
Chromium (ppm)	0.684 ± 0.279	0.330 – 1.380	20
Cobalt (ppm)	0.26 ± 0.164	0.11 – 0.86	19

[a] From formulation
[b] As hydrochloride (thiamine and pyridoxine) or chloride (choline)

TABLE J4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.23 ± 0.060	0.16 – 0.39	25
Cadmium (ppm)	0.05 ± 0.010	0.04 – 0.09	25
Lead (ppm)	0.09 ± 0.016	0.07 – 0.13	25
Mercury (ppm)	<0.02		25
Selenium (ppm)	0.29 ± 0.100	0.18 – 0.49	25
Aflatoxins (ppb)	<5.00		25
Nitrate nitrogen (ppm)[c]	13.91 ± 7.35	4.76 – 36.8	25
Nitrite nitrogen (ppm)[c]	<0.61		25
BHA (ppm)[d]	<1.0		25
BHT (ppm)[d]	<1.0		25
Aerobic plate count (CFU/g)	10 ± 0.0	10.0	25
Coliform (MPN/g)	3.0 ± 0.0	3.0	25
Escherichia coli (MPN/g)	<10		25
Salmonella (MPN/g)	Negative		25
Total nitrosoamines (ppb)[e]	4.81 ± 1.63	2.2 – 9.9	25
N-Nitrosodimethylamine (ppb)[e]	2.7 ± 1.22	1.0 – 6.3	25
N-Nitrosopyrrolidine (ppb)[e]	2.1 ± 0.71	1.1 – 3.6	25
Pesticides (ppm)			
α-BHC	<0.01		25
β-BHC	<0.02		25
γ-BHC	<0.01		25
δ-BHC	<0.01		25
Heptachlor	<0.01		25
Aldrin	<0.01		25
Heptachlor epoxide	<0.01		25
DDE	<0.01		25
DDD	<0.01		25
DDT	<0.01		25
HCB	<0.01		25
Mirex	<0.01		25
Methoxychlor	<0.05		25
Dieldrin	<0.01		25
Endrin	<0.01		25
Telodrin	<0.01		25
Chlordane	<0.05		25
Toxaphene	<0.10		25
Estimated PCBs	<0.20		25
Ronnel	<0.01		25
Ethion	<0.02		25
Trithion	<0.05		25
Diazinon	<0.10		25
Methyl chlorpyrifos	0.139 ± 0.127	0.020 – 0.416	25
Methyl parathion	<0.02		25
Ethyl parathion	<0.02		25
Malathion	0.245 ± 0.243	0.020 – 0.994	25
Endosulfan I	<0.01		25
Endosulfan II	<0.01		25
Endosulfan sulfate	<0.03		25

[a] All samples were irradiated. CFU=colony-forming units; MPN=most probable number; BHC=hexachlorocyclohexane or benzene hexachloride

[b] For values less than the limit of detection, the detection limit is given as the mean.

[c] Sources of contamination: alfalfa, grains, and fish meal

[d] Sources of contamination: soy oil and fish meal

[e] All values were corrected for percent recovery.

APPENDIX K
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via sera or feces from extra (sentinel) animals in the study rooms. These animals and the study animals are subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

During the 3-month studies, serum samples were collected from five male and five female rats and mice at 1 month and the end of the studies; serum samples were collected from an additional five male and five female mice at the end of the study. During the 2-year studies, serum samples were collected from five male and five female sentinel rats and mice at 1, 6, 12, and 18 months and from five male and five female 60 mg/kg rats and mice at the end of the studies. Fecal samples were taken from sentinel mice at 18 months and tested for tested for *Helicobacter* species by polymerase chain reaction. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to BioReliance (Rockville, MD) for determination of antibody titers. The laboratory methods and agents for which testing was performed are tabulated below; the times at which samples were collected during the studies are also listed.

Method and Test	Time of Collection
RATS	
3-Month Study	
ELISA	
PVM (pneumonia virus of mice)	Study start, 1 month, study termination
RCV/SDA	
(rat coronavirus/sialodacryoadenitis virus)	Study start, 1 month, study termination
Sendai	Study start, 1 month, study termination
Immunofluorescence Assay	
Parvovirus	Study start, 1 month, study termination
Sendai	Study termination
2-Year Study	
ELISA	
Mycoplasma arthritidis	Study termination
Mycoplasma pulmonis	Study termination
PVM	Study start, 1, 6, 12, and 18 months, study termination
RCV/SDA	Study start, 1, 6, 12, and 18 months, study termination
Sendai	Study start, 1, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
Parvovirus	Study start, 1, 6, 12, and 18 months, study termination
RCV/SDA	6 and 12 months
Sendai	Study start

Method and Test	Time of Collection
MICE	
3-Month Study	
ELISA	
Ectromelia virus	Study start, 1 month, study termination
EDIM (epizootic diarrhea of infant mice)	Study start, 1 month, study termination
GDVII (mouse encephalomyelitis virus)	Study start, 1 month, study termination
LCM (lymphocytic choriomeningitis virus)	Study start, 1 month, study termination
Mouse adenoma virus-FL	Study start, 1 month, study termination
MHV (mouse hepatitis virus)	Study start, 1 month, study termination
PVM	Study start, 1 month, study termination
Reovirus 3	Study start, 1 month, study termination
Sendai	Study start, 1 month, study termination
Immunofluorescence Assay	
Parvovirus	Study start, 1 month, study termination
LCM	1 month, study termination
MHV	Study termination
2-Year Study	
ELISA	
Ectromelia virus	Study start, 1, 6, 12, and 18 months, study termination
EDIM	Study start, 1, 6, 12, and 18 months, study termination
GDVII	Study start, 1, 6, 12, and 18 months, study termination
LCM	Study start, 1, 6, 12, and 18 months, study termination
Mouse adenoma virus-1	12 and 18 months, study termination
Mouse adenoma virus-FL	Study start, 1 and 6 months
MHV	Study start, 1, 6, 12, and 18 months, study termination
MMV, VP2 (minute virus of mice, viral protein 2)	Study start, 1, 6, 12, and 18 months, study termination
MPV, VP2 (mouse parvovirus, viral protein 2)	Study start, 1, 6, 12, and 18 months, study termination
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	Study start, 1, 6, 12, and 18 months, study termination
Reovirus 3	Study start, 1, 6, 12, and 18 months, study termination
Sendai	Study start, 1, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
EDIM	12 months, study termination
GDVII	Study start, 12 months
Mouse adenoma virus-1	12 months
MCMV (mouse cytomegalovirus)	Study termination
MHV	1 month
MPV	18 months, study termination
Polymerase Chain Reaction	
Helicobacter species	18 months

RESULTS

All test results were negative.

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ISSN 2378-8925